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# PURIFICATION OF POTATO VIRUSES M AND S, AND BIOLOGICAL INTERACTION IN THEIR RESPECTIVE LOCAL LESION HOSTS

by

(C) ALLEN SHU-WING LAU

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled *Purification of Potato Viruses M and S, and Biological Interaction in Their Respective Local Lesion Hosts* submitted by Allen Shu-wing Lau in partial fulfilment of the requirements for the degree of Master of Science.

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Date april 11 1977

*To My Beloved Mother*

*and*

*My Late Father (1911-1972)*

# ABSTRACT

Interaction between potato virus M (PVM) and potato virus S (PVS) was investigated using purified preparations of intact particles and ribonucleic acids (RNA's) in their respective local lesion hosts. The purification of PVM and PVS by differential precipitation with polyethylene glycol and sodium chloride resulted in increases in the virus yield up to 10-fold (330-350 mg virus per kg of infected leaves) over those obtained by the other methods reported in literature. The values of Absorbance 260/280 were found to be 1.46±0.04 and 1.48±0.03 for purified PVM and PVS respectively. The values of sedimentation coefficent and buoyant density of PVM and PVS were 108 and 148S, and 1.35 and 1.34 gm/ml respectively.

Inoculations of *Phaseolus vulgaris* L. cv. 'Red Kidney' or *Chenopodium quinoa* Willd. with a mixture of purified PVM and PVS respectively gave rise to marked increases in the local lesion number incited by a test virus, i.e. PVM or PVS. Neither PVM nor PVS mixed as a counterpart of a given test virus was recovered from the inoculated leaves of the non-host plant after mixed application. Approximately 10 to 30% increases in local lesion number incited by the test viruses were also obtained in the presence of the coat-proteins of the counterpart in the inoculum. Interaction of the viruses was not found when simultaneous separate inoculations were made onto the upper and lower epidermal layers of the test plants.

In the presence of the viral RNA of the counterpart, the

numbers of local lesions due to intact virus decreased 30 to 60%. Similarly, experiments with inoculum of mixed viral RNA's yielded 10 to 30% inhibition in the infectivity of the test viral RNA. This result suggested that there was no genetic interaction between these two viruses even at RNA level. The activity of the endogenous ribonuclease in the purified virus preparations was reduced by diluting the intact viral counterparts before mixing with the RNA preparations or by adding yeast-RNA to the viral RNA preparations when intact viral counterparts were mixed.

The results suggested that the stimulation of infection by a mixture of PVM and PVS was achieved possibly through the physical interaction of the particles between the intact viruses and even to a limited extent through an interaction with coat-protein of the counterpart. Thus, it was concluded that this type of interaction found between PVM and PVS in their respective local lesion host was a unique phenomenon different from any known interactions between different plant viruses.

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# LIST OF ABBREVIATIONS

| Abbreviation | Vernacular name of the virus |
|---|---|
| AMV | alfalfa mosaic virus |
| CBV | chicory blotch virus |
| CLV | carnation latent virus |
| CMMV | cowpea mild mottle virus |
| CVB | chrysanthemum virus B |
| CV2 | cactus virus 2 |
| FMV | fressia mosaic virus |
| HLV | hop latent virus |
| LSV | lily symptomless virus |
| NLV | narcissus latent virus |
| PAMV | potato aucuba mosaic virus |
| PLV | passiflora latent virus |
| PMV | poplar mosaic virus |
| PSV | pea streak virus |
| PVA | potato virus A |
| PVM | potato virus M |
| PVS | potato virus S |
| PVX | potato virus X |
| RCVMV | red clover vein mosaic virus |
| STNV | satellite tobacco necrosis virus |
| TEV | tobacco etch virus |
| TMV | tobacco mosaic virus |
| TNV | tobacco necrosis virus |
| TYMV | turnip yellow mosaic virus |
| WBMV | white bryony mosaic virus |

# CHAPTER I

## INTRODUCTION

Although the taxonomy of viruses is a highly controversial subject (Gibbs, 1969; Lwoff and Tournier, 1971), potato viruses M and S are now classified as members of the carlavirus group (Harrison et al., 1971), and this sigla grouping has been approved recently by the International Committee on the Taxonomy of Viruses (Fenner, 1976). The carlavirus group includes carnation latent virus (CLV) as its type member, and additional 11 members such as cactus virus 2 (CV2), chrysanthemum virus B (CVB), cowpea mild mottle virus (CMMV), lily symptomless virus (LSV), narcissus latent virus (NLV), passiflora latent virus (PLV), pea streak virus (PSV), potato virus M (PVM), potato virus S (PVS), red clover vein mosaic virus (RCVMV), and white bryony mosaic virus (WBMV). There are four more possible members suggested on the basis of partial characterization of the viruses. They include chicory blotch virus (CBV), fressia mosaic virus (FMV), hop latent virus (HLV), and poplar mosaic virus (PMV) (Brandes, 1964; Harrison et al., 1971). The members of the group are characterized by a particle length of 620-690 nm, straight to slightly flexuous rods, containing about 6% ribonucleic acid (RNA) (Gibbs, 1969; Harrison et al., 1971). Their longevity *in vitro* is only a few days at 20°C, the thermal inactivation point (TIP) is between 55°-70°C, and the dilution end point (DEP) is $10^{-2}$-$10^{-4}$. They induce mild or no symptoms and have a narrow host range. Besides mechanical transmission, the viruses of this group may be transmitted by aphids. Their serological relationships range from nil to distant (Kassanis, 1955, 1956; Bagnall et al., 1956a,b; Rozendaal and van Slogteren, 1958; Bagnall et al., 1959; van Slogteren et al., 1962; Hakkaart et al., 1962; Brandes and Wetter, 1963-64; Wetter, 1967; Varma et al., 1970; Bos et al., 1972).

Soon after the turn of this century, Canada acquired an international reputation for high quality seed potato production through constant efforts in disease control and research in addition to climatic conditions suitable for such a purpose. Seed potato certification,started in Canada in 1913 by the National Plant Quarantine Act of 1912, aimed to restrict the spread of potato diseases across countries or continents (Rieman, 1956). By 1922, the certified acreage for seed potato production in Canada was 6,642 acres. Large scale serological and indicator plant assays began in 1952 to maintain seed potato stocks free from potato virus X (PVX) infection (Munro, 1954). Virus-tested potato stocks were established in British Columbia (Stace-Smith and Mellor, 1967, 1968a,b; Mellor and Stace-Smith, 1967, 1970, 1971; Wright, 1970; Wright and Mellor, 1976), and were introduced into elite seed farms in Eastern Canada in 1969 (MacKinnon and Bagnall, 1972). Approximately 85% of the certified seed potato acreage is in Prince Edward Island (P.E.I.) and New Brunswick (Seed Potatoes from Canada, 1975), and more than 75% of seed potatoes in Canada are grown annually in P.E.I. to meet the needs of both domestic and export markets (MacKinnon et al., 1972).

In Alberta today, the southern irrigated areas and the central region from Lacombe to Edmonton are the two major commercial potato farm lands (Holmes et al., 1976). The Alberta Seed Potato Improvement Program (ASPIP) was established in 1971 to supply the growers with disease-tested seed potatoes (Letal and Hiruki, 1976). Under this program seed potatoes, in particular Elites I and II, have been grown in isolated fields at Lacombe and Peers in Central Alberta. In 1976, while Alberta had 17,000 acres under potato cultivation, a 12% increase in yield was reported in the province (Fruit, Vegetable and Honey Crop and Market Report, 1976).

In 1976, Canada had 283,600 acres under potato production as compared to 260,000 acres in 1975 (Fruit, Vegetable and Honey Crop and Market Report, 1976). Of this, 65,000-71,000 acres were involved in the production of seed potatoes, and of which about 45,000 acres were for export purpose. Elite seed potatoes are maintained continuously by clonal selection, serological tests, indicator plant assays and other necessary tests to assure that they are free of tested viral, fungal or bacterial infection. At present, about 46 potato varieties are licensed for sale as seed potatoes (Seed Potatoes from Canada, 1975), and between 1964-1973, the annual export amounted to 125,000 tons.

Although Canadian seed potatoes have been exported to over 20 countries, the largest seed potato exporter in the world is the Netherlands. Van der Zaag (1972) reported that between 1965-1970 about 270,000 tons of certified seed potatoes were exported to more than 50 countries. Other European countries with significant export of seed potatoes are the United Kingdom, the Republic of Ireland, Denmark and France.

Control of viruses in seed potatoes has had a long history. There are three principle methods, which have been used separately or in combination for better results: (1) varietal resistance, (2) thermotherapy, and (3) meristem tip culture.

Bagnall et al.(1956b) reported that certain cultivars including 'Saco', 'Alpha', 'Sebago', 'Voran' and 'Seedling X927-3' were found free from PVS infection by serological tests. Evidence of resistance to PVS in 'Saco' was reported also elsewhere (Alfieri and Stouffer, 1957; Bagnall et al., 1959; Alfieri, 1960; Bagnall and Young, 1960, 1968, 1972; Bagnall, 1965; Zadina, 1971). However, Larson and Oshima (1959) later found that when PVS was introduced to 'Saco' plants by top-graft inoculation the virus passed through the stem tissues into the roots where it could be detected. Baerecke (1967a) and Vulić and Hunnius (1967a) also reported transmission of PVS from infected scion to 'Saco' plants. Bagnall and Young (1960, 1969, 1972) hypothesized that resistance of 'Saco' potato was controlled by a single gene in the homozygous recessive condition, i.e. ssss being resistant to PVS while potato with a dominant gene 'S' is susceptible. Scholz (1964) obtained a PVS-resistant hybrid by crossing 'Ora' and 'Schwalbe' potatoes. Baerecke (1967b) and Makarov (1975) found that the resistance to PVS of a Bolivian clone, *Solanum tuberosum andigena* 'Huaca ñahui', Plant Introduction 258907, was due to the presence of a single dominant gene $N_s$. On screening 43 tuber-bearing *Solanum* species, Bagnall (1972) discovered that *S. microdontum* was resistant to both PVM and PVS.

Heat treatment has been a useful and an easily applicable therapeutic method for controlling virus and mycoplasma diseases of plants. Effective treatment temperature and duration may vary from 35° to 54°C and from hours to weeks. Two kinds of potato materials have been used: the dormant tubers and young growing plants. The treatment of dormant tubers may rely strictly upon relatively simple heat-inactivation of the virus, however, hot-air-treatment of vigorously growing plants is more widely used.

The discovery of the frequent absence of tobacco mosaic virus (TMV) at the growing tip of infected plants (Limasset and Cornuet, 1949) consolidated Morel's (1948) pioneer work on the meristem tip culture technique to free plants from viruses. Hollings (1965) defined a meristem tip culture as an aseptic culture of apical meristem dome plus the first pair of leaf primordia, which is about 0.1-0.5mm long in different plants. Meristem tip culture alone often gives rise to virus-free clones, and is especially valuable when heat treatment fails (Bawden et al., 1950) or where plants cannot withstand heat. By growing the excised apical meristem (0.1-0.25mm) of sprouts from 'King Edward' and 'Arran Victory' infected with PVM and PVS, Kassanis (1957) obtained a limited number of virus-free plants. Similarly, Quak (1961), Yora and Tsuchizaki (1962), and Huth and Bode (1970) also produced few PVS-free potato plants. Morel and Muller (1964) eradicated PVS and PVX from 'Red Pontiac' and 'Eersteling' potatoes using the same technique with improved medium. Pett (1974) claimed, using meristem tip cultures, 84.6% of 'Axila' and 'Sperber' were made free from PVS, while 71.4% of 'Meise' were also free from PVM and PVS.

However, simple heat treatment or meristem tip culture alone sometimes fails to produce virus-free potato plants. Virus-free nuclear stocks can be obtained more efficiently by combining thermotherapy and meristem tip culture techniques (Quak, 1972). Thomson (1958a) obtained some healthy potato plants by culturing PVS-infected shoot apices at 30°-38°C in darkness for 7-110 days, then excising and subculturing the shoot apices (1-2 cm). Similarly, Morel (1964) cultured meristem tips of sprouts from PVS-infected tubers germinated in darkness at 37°-38°C to obtain virus-free plants. Stace-Smith and Mellor (1968a,b) also succeeded in eliminating both PVS and PVX from infected 'White Rose' by growing the infected plants at 33°-37°C (with soil temperature at 30°- 32°C) for up to 7 months and culturing the excised axillary buds (0.3-1 mm, including 2-3 leaf primordia) on Murashige and Skoog's (1962) liquid medium. These techniques were soon applied to other infected stocks so that over 40 cultivars of potato licensed for sale in Canada were freed from PVS and PVX (Mellor and Stace-Smith, 1970; Wright, 1970; Wright and Mellor, 1976). Wright (1970) reported that PVS- and PVX-free 'Netted Gem' (Russet Burbank) and 'White Rose' seed potatoes increased their yield by 11-38% in British Columbia, Oregon and California. In fact, Shepard and Claflin (1975) stated that the only serious attempt being made in North America to produce PVS- and PVX-free certified seed potatoes at the grower level is in British Columbia, Canada.

Huth and Bode (1970) eliminated PVS from infected German potato varieties by heat treatment of tubers and meristem tip cultures, followed by grafting of plantlets onto tomato plants. In Finland, Tapio (1972) similarly reported an 80% recovery of potato plants free from PVS and PVX from apical meristems. Macdonald (1973) in Scotland also recovered 58% of potato plants free from PVS and PVX when tested serologically.

Although much effort has been made in establishing virus-tested nuclear stocks, reinfection can occur. In nature, PVS can be transmitted by the aphid, *Myzus persicae* Sulz. (Bode and Weidemann, 1971; MacKinnon, 1974). After the introduction of virus-tested seed potatoes, 3-57% reinfection by PVS and PVX occurred in P.E.I. during 1969-1972, depending on the closeness of virus source and varietal susceptibility (MacKinnon et al., 1972; MacKinnon, 1974).

Nevertheless, there were two- to threefold increases in yield globally during the period 1935-1965 through improved health of seed potato stocks (Hollings, 1965). Shepard and Claflin (1975) reported three United States and one Canadian growing regions had recorded yield increases of 14-37% by using virus-tested seed potatoes. Between 1962-1973, 23% and 30% yield increases were reported from potato growing areas in the temperate and tropical zones in the world respectively (van der Zaag, 1976). Although the average yield of potatoes has been 13.3 tons/hectare, 95 tons/hectare has been achieved in experimental fields in the Netherlands. The survey from 1962 to 1973 indicated that the total acreage for world potato production will decrease continuously because of constant increase in yield due to the availability of disease-tested seed potatoes.

Bagnall et al. (1956a,b) determined serologically that PVM and PVS had a relatively small fraction of the antigenic determinants in common. On the basis of comparative serological studies on PVM, PVS and CLV, Kassanis (1956) and Bagnall et al. (1956b, 1959) suggested that these three viruses might have had a common ancestry. Van Slogteren et al. (1962) and Hakkaart et al. (1962) proposed that the mutual degrees of serological relationships between PVM, PVS, CLV and CVB could be arranged as PVM-CLV-CVB-PVS in order of relatedness.

PVS was found with PVM in most commercial stocks of 'King Edward' potatoes (Bagnall et al., 1956b, 1959; Kassanis, 1956, 1960, 1961a; Rozendaal and van Slogteren, 1958; Howard and Wainwright, 1960; Bawden and Kassanis, 1964; Beemster and Rozendaal, 1972). Kassanis(1956) and Bawden and Kassanis (1964) reported that PVM and PVS could multiply, without interfering with each other, to much the same extent as if each of them were alone. Cross-protection, a phenomenon whereby serologically related viruses are mutually antagonistic and plants fully infected with one resist invasion by another, was not clearly observed between these two viruses (Kassanis, 1956; Bagnall et al., 1956b, 1959; Rozendaal and van Slogteren, 1958; Bawden and Kassanis, 1964; Beemster and Rozendaal, 1972), but different degrees of interference were reported between their strains by Rozendaal and van Slogteren (1958). These results suggest that the serological difference between PVM and PVS resulted in the apparent loss of their capability to interfere with each other. Bagnall et al. (1956b) found that a mixed inoculation of PVM and PVS resulted in the quick appearance of the chlorotic local lesions on *Datura Bernhardii* Lund. and *D. metel* L., while PVS alone did not incite symptoms on either of the hosts, although it infected *D. metel* systemically.

On the other hand, Ambrosov and Sokolova (1973) reported that secondary infection with PVS significantly reduced infection by

PVM in the potato plants when determined serologically. Hunnius (1972), however, reported that the multiplication of PVS was inhibited by the secondary infection with PVM and later PVS was replaced completely. Howard and Wainwright (1960) and Bawden and Kassanis (1964) suggested that in mixed infections by PVM and PVS, the resulting symptoms depended largely on the virulence of the PVM strain. Hiruki (1970) found that there was a strong inhibitory effect by PVS on PVM infection in its local lesion host, *Phaseolus vulgaris* L. cv. 'Red Kidney', when a mixed inoculum in sap was used.

Therefore, the biological interaction between PVM and PVS remains as a controversial topic. One of the reasons for this was the lack of reliable bioassay systems for these viruses in the past. To determine the presence or absence of interference or interaction between the two viruses it is essential to analyze a mixed infection with highly purified viruses by the local lesion method or other quantitative techniques. In the past there were no such careful analyses made on these viruses. Although PVM and PVS have been known since 1923 and 1951 respectively, our understanding of their physicochemical properties is still fragmentary.

In the present study, investigations have been mainly directed towards the purification of potato viruses M and S in large quantities with improved yields. Using the highly purified virus preparations the physicochemical and biological properties of PVM and PVS have also been studied. The biological interaction of purified PVM and PVS was investigated at intact particle and RNA levels in their respective local lesion hosts. The effects of endogenous ribonuclease, coat-protein and yeast-RNA on the viral interaction have been included as well in this study.

# CHAPTER II

# LITERATURE REVIEW

## A. Potato Viruses M and S

Serious confusions in nomenclature occurred when earlier workers assigned different names for the same virus on the basis of symptoms, which might vary in severity under different conditions. A virus disease, believed to be caused by PVM, was first encountered by E. S. Schultz in 1923 and may serve as a typical example in this respect. Symptoms incited by PVM in 'Green Mountain' was first described under the name of leafrolling mosaic (Schultz and Folsom, 1923). Since then at least nine more synonyms have been assigned to this same virus (Rozendaal and van Slogteren, 1958; Wetter, 1972) as listed in the following: potato virus 7 (Johnson, 1927), potato paracrinkle virus (Salaman and Le Pelley, 1930), potato interveinal mosaic virus (McKay and Dykstra, 1932), potato virus E [unpublished thesis of F. C. Bawden (Smith, 1933)], Kartoffel-Rollmosaik-Virus (Kohler, 1935), Solanum virus 7 (Smith, 1937), Solanum virus 11 (Smith, 1937), *Marmor angliae* Holmes (Holmes, 1939) and Kartoffel-K-Virus (Kohler, 1941). Bagnall et al. (1956b) designated the "interveinal mosaic virus (IVM factor)" of the 'Irish Cobbler' potato as "potato virus M", which at last won its universal acceptance.

PVS was accidentally discovered in 1948 at the Laboratory for

Flowerbulb Research at Lisse, the Netherlands, directed by E. van Slogteren during serological tests for potato virus A (PVA). Antiserum produced by using a supposedly pure PVA source from 'Light Industrie' not only reacted non-specifically with PVA alone, but also with an unknown viral antigen obtained from 'Light Industrie', 'Dark Industrie', and 'Bintje' regardless of the presence of PVA (van Slogteren, 1955a,b; Rozendaal and Brust, 1955). The field occurrence of this virus was first reported in 1951 by de Bruyn Ouboter (1952), and later was named as potato virus "S" in honor of its original discoverer, E. van Slogteren (Rozendaal, 1954; MacLeod, 1962).

The host range of potato viruses M and S includes several species in the families of Amaranthaceae, Chenopodiaceae, Leguminosae, and many species belonging largely to the Solanaceae (Bagnall et al., 1956b, 1959; MacLeod, 1962; Wetter, 1971, 1972). *Lycopersicon esculentum* Mill. (tamato) is immune to PVS, but systemically susceptible to PVM without producing visible symptoms. It may, therefore, be used as a filter plant to separate PVM from a mixed-inoculum of PVM and PVS (Rozendaal and Brust, 1955; Bagnall et al., 1956b; Kassanis, 1956; Vulić and Hunnius, 1967b; Horváth, 1972, 1973). Hiruki (1970) found that French bean 'Red Kidney' was also immune to PVS, suggesting that PVM could be separated from double-infected samples with subsequent subculture in tomato. Bagnall et al. (1956b, 1959) suggested that *Nicotiana debneyi* Domin. could be used as a test plant for the isolation of PVS since it is systemically susceptible to PVS while locally susceptible to PVM.

According to the cryptogram proposed by Gibbs et al. (1966) and Gibbs and Harrison (1968), PVM and PVS are described as "*/* : */* :

E/E : S/Ap" and "R/1 (Hinostroza-Orihuela, 1973) : */* : E/E : S/Ap" [Type of nucleic acid (NA)/Strandedness : MW of NA/% NA : Outline of particle/Shape of nucleocapsid : Hosts/Vector] respectively, where E = elongated with parallel sides, ends not rounded, S = seed plant, Ap = aphid, R = ribonucleic acid, and 1 = single. Both viruses are straight to slightly flexuous filamentous particles of approximately 650 x 12 mm in size (Wetter and Brandes, 1956; Brandes et al., 1959; De Bokx, 1969; Tu and Hiruki, 1970; Hiruki and Shukla, 1973). PVM has a TIP of 65°-71°C (Wetter, 1972) while PVS's is 55°-60°C (Bagnall et al., 1956b; Wetter, 1971). Both viruses have a DEP of $10^{-2}$-$10^{-4}$ (Bawden et al., 1950; Bagnall et al., 1956b; Wetter, 1971, 1972; Hiruki, 1973, 1975a), and their longevity *in vitro* is only 3 to 4 days at 20°C. Although Kassanis (1956, 1961a) failed to transmit PVM in 'King Edward' of the Rothamsted stock by aphids, Wetter and Völk (1960) succeeded in transmitting German isolates of PVM by *M. persicae* in other potato cultivars. It appears that different strains of PVM vary considerably in their aphid transmissibility. Bode and Weidemann (1971) reported that the rate of transmission by *M. persicae* ranged from 1 to 82% with five PVM strains. Similarly, despite earlier reports to the contrary (Rozendaal and Brust, 1955; Kassanis, 1956) various isolates of PVS were transmitted in the range of 3.4% to 42% by *M. persicae* (Bode and Weidemann, 1971; MacKinnon, 1974). However, PVM and PVS were not transmitted via true seeds of various infected *Lycopersicon* species (Horváth, 1973) or potato cultivars (Shepard and Claflin, 1975; Goth and Webb, 1975). These viruses are mainly maintained from generation to generation through the tubers of infected potato plants. Before disease-tested seed potatoes were available, farmers in the Netherlands tended to retain smaller tubers from the previous crop for seed purpose, and this practice helped the spread of these viruses (Rozendaal and Brust, 1955).

In potatoes, PVM incites symptoms ranging from very slight (e.g. 'King Edward') to severe (e.g. 'Arran Victory'). 'King Edward' infected with PVM is usually symptomless and occasionally shows slight ruffling and paling of leaves, while in other cultivars, various diffused interveinal mottling, mosaic, crinkling and rolling symptoms in leaves, and dwarfing of the plant are observed (Bagnall et al., 1956b). The symptoms incited by PVS in potatoes are downward rolling, rugosity, slight veinbanding, chlorosis of leaves and stunting of shoots. Chmulev (1974) and Ambrosov and Schchutskaya (1975) reported infections by these two viruses resulting in marked decreases in pigmentation and photosynthetic activity in potato plants, but with increased respiration and oxidase activity.

## B. Distribution and Economic Importance

Potato ranks fifth as a staple crop for human consumption following wheat, rice, maize and barley (van der Zaag, 1976). The total world potato crop production at present has amounted to 290 million tons and of these, 150, 100, 30, 8 and 2 million tons have been used for human consumption, stockfeed, seed, starch- and alcohol-productions respectively. In North America, 80% of the total potato production have been used for human consumption and 50% of which are in processed forms. In Europe, however, 50% of the potatoes produced have been used as stockfeed.

PVM and PVS are of economic importance because they are widely distributed wherever potatoes are grown. In North America, Schultz (1951) reported that PVM reduced the yield of three potato varieties by 13-18% in Maine. PVM was also found in Oregon (McKay and Dykstra, 1932; Bagnall et al., 1956a,b, 1959; Kahn et al., 1967). Infection by PVS did not affect the number of 'Sabago' tubers but reduced the tuber weight (Lim et al., 1966). Other reports of PVS occurrence included Wisconsin (Rozendaal and Brust, 1955; Bagnall et al., 1956a,b, 1959: Larson and Oshima, 1959), California (Gold and Oswald, 1955), Oregon (Vaughan and van Slogteren, 1956), New York (Alfieri, 1960), Michigan (Lim, 1967), and others (Kahn et al., 1967).

In Canada, PVM was isolated from 'King Edward' and some other cultivars in Ontario and New Brunswick (MacLeod, 1939, 1962; Bagnall et al., 1956a,b, 1959), and more recently in Alberta (Hiruki 1970, 1973; Tu and Hiruki, 1970). Wright (1970) estimated that losses due to PVS in British Columbia ranged from 10 to 30%, especially when other viruses such as PVM or PVX were present. Occurrence of PVS was reported in New Brunswick (Bagnall and Bradley, 1955; Bagnall et al., 1956a,b, 1959), British Columbia (Stace-Smith and Mellor, 1968a,b), P.E.I. (MacKinnon, 1974), and Alberta (Hiruki, 1975a).

In Central and South American countries including Mexico, Guatemala, Haiti, Colombia, Ecuador, Peru, Bolivia and Chile, the overall incidence of PVM and PVS infectons in various potato cultivars was about 2 and 14% respectively (Kahn and Monroe, 1970). McKee (1964) established the presence of PVS in Peruvian potatoes and Mendoza (1967) found that about 17% of them were infected by PVS. More recently, Hinostroza-Orihuela (1973) revealed an average of 28%

infection with PVS in different Peruvian cultivars. In Argentina, Pontis and Feldman (1963) reported the first record of PVM infection in the country.

Although PVM is not as widespread as PVS in Western Europe, its presence has been reported in England (Salaman and Le Pelley, 1930; Bawden et al., 1950; Kassanis, 1956, 1960, 1961a), West Germany (Köhler, 1941; Vulić and Hunnius, 1967b), France (Payen and Madec, 1957), the Netherlands (Rozendaal and van Slogteren, 1958), Italy (Lovisolo and Benetti, 1959), U.S.S.R. (Kolobaev, 1963), East Germany (Scholz, 1965a), Rumania (Ghena, 1970), and Poland (Chrzanowska, 1973, 1976). There are strong indications that PVM is now the most important virus of potatoes in Eastern Europe including U.S.S.R. (Hiruki, personal communication). Yield losses due to PVS were estimated to be 10-20%, depending on potato cultivar as well as virus strain (Rozendaal and Brust, 1955; van Slogteren, 1955c; Vaughan and van Slogteren, 1956; Kameraz and Shcherbakova, 1957; Beemster and Rozendaal, 1972). In the Netherlands, many potato cultivars have been much more severely infected with PVS than with PVX (Rosendaal and Brust, 1955; Rozendaal and van Slogteren, 1958). Widespread occurrence of PVS in various potato cultivars has also been reported in West Germany (Köhler, 1953, 1955; Wetter and Brandes, 1956), Scotland MacArthur, 1956), France (Spire et al., 1967), Denmark (Kristensen, 1956), Finland (Aura, 1957; Tapio, 1972), Italy (Lovisolo and Benetti, 1959), England (Kassanis, 1961a), U.S.S.R. (Kamerag and Shcherbakova, 1957), Czechoslovakia (Jermoljev and Průša, 1958; Šíp and Havlová, 1969; Šíp, 1974), East Germany (Scholz, 1962, 1965a, b), Bulgaria (Bailova-Yankulova, 1966), Rumania (Ghena, 1967, 1970), and Hungary (Horvâth, 1972 ).

Similarly, PVM and PVS were also found in Asia. Both viruses occurred in Japan (Oshima and Sato, 1965; Horio et al., 1969; Horio and Kumoi, 1975). Horio et al. (1969) reported that PVM infection in seed potatoes ranged from 11 to 100% in seven cultivars tested. PVM and PVS were present in a number of Korean potato seed stocks (La, 1974). Several PVM strains also were detected in Taiwan (Lee, 1971). The spread of PVS in India was studied by Ganguly (1963) and by Upreti and Nagaich (1969).

Despite the known occurrence of PVS (Sampson and Taylor, 1968), PVM has not yet been found in Australia (D.S. Teakle, personal communication). PVS has been identified serologically in three potato cultivars in New Zealand (Thomson, 1959). In South Africa, Herold (1967) found that 16% of various potato cultivars were infected with PVS.

## C. Purification

Purification of the members of the carlavirus group has always been difficult in the past because of the low virus concentration in host tissues and losses due to aggregation of virus particles during isolation processes. Filamentous viruses are known for their frequent formation of insoluble aggregates, especially during concentration steps (Clark and Lister, 1971; Hödrejärv et al., 1970; Paliwal and Tremaine, 1976). These difficulties have considerably limited studies of physicochemical properties of the viruses belonging to the carlavirus group.

Polyethylene glycols (PEG) (trade name "Carbowax" is referred to solid PEG with MW over 1000 dalton), uncharged linear polymers having a general formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, are chemically inert and their melting point, viscosity and aqueous solubilizing action all increase with increasing molecular weight (McClelland and Bateman, 1945). McClendon (1954) first reported that 20% (w/v) or higher Carbowax 4000 (MW 2400) together with 1 M sodium chloride (NaCl) precipitated the "ultraviolet absorbing materials" from his chloroplast preparations. He signified that this discovery might provide a new tool in the study of nucleoproteins. Attempts were made initially to purify and concentrate viruses using aqueous two-phase systems of sodium-dextran-sulphate and PEG (Albertsson, 1960; Philipson et al., 1960). It was noted that the concentration of NaCl was significant in the system and it directly affected the final concentration of the virus in one phase or the other.

Hebert (1963) directly applied PEG 6000 and NaCl for precipitating plant viruses instead of the laborious conventional methods (reviewed by Steere, 1959). The two filamentous plant viruses he studied were precipitated by using 4% (w/v) or less PEG while the two spherical viruses were precipitated by 8% (w/v) PEG. The effect of PEG concentration on isometric virus precipitation was also studied later by Lastra and Munz (1969), who found a 90% recovery of the infectivity of squash mosaic virus by using 6% PEG 6000, while 4 and 2% PEG recovered only 70 and 40% of its total infectivity respectively. Venekamp and Mosch (1963; 1964a,b,c) and Leberman (1966) examined various concentrations of PEG 6000 and NaCl in cellulose column chromatography for virus purification. The virus could then be eluted with a buffer solution containing neither PEG nor NaCl. Using the same technique Clark (1968) tried to precipitate alfalfa mosaic virus (AMV) with 8% (w/v) PEG 20000 and fractionated it on a kieselguhr column by washing with a 10% (w/v) nonlinear decreasing gradient solution of PEG 6000. Adomako et al. (1974) chromatographed cocoa necrosis virus that had been precipitated with 10% PEG 6000 on Celite and gel- (Sephadex G200, Sepharose 4B) filtration columns with decreasing concentrations of PEG (5, 3, 1, 0%) as eluent. This chromatographic purification was carried out with a belief that plant viruses could be precipitated and redissolved at different PEG and NaCl concentrations.

Investigation was made into the centrifugation of PEG-precipitated plant viruses through a reverse concentration PEG solubility gradient (10-0%), which was stabilized by a positive sucrose density gradient (5-30%) containing 0.15 M NaCl, for the determination of the minimal concentration of PEG and NaCl required for the precipitation of the virus or its components (Clark, 1970; Clark and Lister, 1971). In studying wound tumor virus, Reddy and Black (1973) reported both PEG and NaCl concentrations were very critical since they had a marked effect upon virus yield and specific infectivity.

Soon after Hebert's (1963) discovery, the PEG-NaCl precipitation procedure was widely adopted for the purification of not only other plant viruses but also animal viruses (Kanarek and Tribe, 1967; Wagner et al., 1970; McSharry and Benzinger, 1970; Freidmann and Haas, 1970) and Bacteriophages (Yamamoto et al., 1970). Recent reports involving PEG-NaCl purification of plant viruses are: pea seed-borne mosaic virus (Knesek et al., 1974), carrot thin-leaf virus (Howell and Mink, 1976), ryegrass mosaic virus (Paliwal and Tremaine, 1976), soybean dwarf virus

(Kojima and Tamada, 1976), tobacco mosaic virus (cowpea strain) (Whitfeld and Higgins, 1976).

## D. Interaction Between Different Plant Viruses

Information on plant virus interactions is scattered through a tremendously large volume of literature and has been reviewed frequently (Price, 1940, 1964; Bennett, 1951, 1953; Ross, 1957, 1959, 1974; Kassanis, 1963; Best, 1965; Köhler, 1969; Matthews, 1970; Loebenstein, 1972a,b; Gibbs and Harrison, 1976; Dodds and Hamilton, 1976). In dealing with virus diseases occurring in nature it is not unusual to find double, triple or even quadruple virus infections in plants. While strains of a virus are mutually antagonistic, both synergism and antagonism commonly occur between different viruses. These interactions are often expressed in symptomatology and/or results in an abnormal increase in one of the interacting viruses.

Kassanis (1963) and Price (1964) interpreted interference as a phenomenon whereby a virus or viral component interferes with the multiplication of an unrelated virus or its disease-producing capacities, and that cross protection is a term designated to interference between strains of a virus only. In addition, these two phenomena differ in such a way that cross protection is specific, often almost complete, relatively long lasting, and frequently reciprocal, but interference is non-specific, incomplete, short lasting and one directional. However, Loebenstein (1972b), with a more inclusive view after considering recent progress made in this area of research, defined the interference of plant viruses as "a phenomenon whereby the infection process of one virus interferes with the multiplication of another virus or strain; or whereby a non-multiplying substance interferes via a host-mediated process, requiring the transcription mechanism of the cell, with the multiplication of a virus".

In this review more emphasis will be given to the interaction between different viruses. Firstly, in synergistic interaction, a double infection by TMV and PVX, which resulted in severe streak symptom in tomato plants, is perhaps the first example that is well-known in plant virology (Dickson, 1925; Vanterpool, 1926). In other double infections, severe symptoms also resulted from a combination of TMV and tobacco etch virus (TEV) (McWhorter and Price, 1949), potato aucuba mosaic virus (PAMV) and AMV (Oswald et al., 1955), or TMV and cucumber mosaic virus (Garces-Orejuela and Pound, 1957; Marrow and Migliori, 1971). Development of a new symptom such as local lesions, which was not produced by neither of individual viruses, was found upon concurrent infection with a mixture of TMV and PVX (Sadasivan, 1940; Ross, 1959). In mixed infection, the concentration of interacting viruses is also affected. For example, the concentration of dodder latent mosaic virus (DLMV) greatly increased with no change in the concentration of the secondary viruses, when tomato plants infected with DLMV was superinfected with TMV or TEV (Bennett, 1949). Similarly, an increase of PVX concentration up to 10-fold was detected in the presence of PVY in potato and tobacco plants; the increase was still higher in simultaneous inoculation than in separate inoculations made in sequence (Ross, 1950; Rochow and Ross, 1954, 1955; Thomson, 1958b; Goodman and Ross, 1972). Concurrent inoculation with TMV and PVX or using TMV as a challenge virus, an increase up to 5-fold in

PVX was reported (Zachos, 1957; Thomson, 1961). TMV concentration in barley was much higher in the presence of barley stripe mosaic virus than in single infection with TMV (Hamilton and Dodds, 1970; Dodds and Hamilton, 1972).

Secondly, a number of reports pertaining to antagonistic effect of mixed infection exist, in which the viruses and procedures similar to those mentioned above were used. In antagonistic infection with mixed viruses, reduction in virus concentration was often noted. For example, infection with TEV established in potato or tobacco plants interfered with the secondary infection with PVY or henbane mosaic virus, and in concurrent inoculation, the systemic infection by the latter two viruses was suppressed or almost completely replaced by TEV even if they had first become established in the hosts (Bawden and Kassanis, 1945). In simultaneous infection with PVX and PVY, the latter decreased as the amount of PVX increased (Ross, 1950, 1957; Hutton, 1952). When TMV and PVX were inoculated simultaneously on tomato plants, a decrease of 20 to 65% in TMV concentration was found (Rochow, 1954; Rochow and Ross, 1954; Zachos, 1957). The speed of lesion development and/or the size of lesions incited by one virus in the established presence of another one decreased significantly (Price, 1935; McKinney, 1941; Bode, 1948; Ross, 1953; Nitzany and Cohen, 1960; Nitzany and Sela, 1962; Ford, 1967; Wenzel, 1971; Kassanis et al., 1974; Hiruki, 1975b). By contrast, simultaneous inoculation with TMV and PVX resulted in increases in the lesion-size of TMV, whereas if PVX was pre-inoculated, the resulting effect was negligible (Ross, 1961).

An unprecedent type of interaction was reported to occur between satellite tobacco necrosis virus (STNV) and tobacco necrosis virus (TNV) (Kassanis, 1968). In this system, the multiplication of STNV was completely dependent on the presence of TNV, and in mixed inoculation in tobacco or bean plants the concentration of TNV decreased depending on the amount of STNV present in the inoculum (Kassanis and Nixon, 1961; Kassanis, 1962; Kassanis and White, 1972).

The studies of interaction between viruses have recently been carried out in protoplasts using both strains (Otsuki and Takebe, 1974) and unrelated viruses (Otsuki and Takebe, 1973, 1976; Takebe et al., 1975).

Lastly, another feature of interference is the interaction of an insect non-transmissible virus which becomes eligible in the presence of an insect transmissible virus. Clinch et al. (1936) were the first to find that PAMV became transmissible by aphids in the presence of PVA; and later PAMV was discovered also transmitted together with PVY (Kassanis, 1961b). Smith (1945, 1946) reported that tobacco mottle virus could only be transmitted by *M. persicae* together with the aphid dependent tobacco veindistorting virus present in the double infected tobacco plants.

# CHAPTER III

# PURIFICATION AND PHYSICOCHEMICAL PROPERTIES

## INTRODUCTION

The purification of PVM and PVS was initially attempted by van Slogteren (1955) and Rozendaal and van Slogteren (1958). Their method involved extraction of sap in the presence of sodium bisulphite and potassium cyanide, clarification of the sap with chloroform, and isolation of the viruses by differential centrifugation. Wetter (1960) then purified both viruses, after clarification of sap with ether and carbon tetrachloride, by differential and rate-zonal density gradient centrifugation followed by dialysis. Albrechtová and Klír (1968) reported that PEG 4000 was suitable for the precipitation of PVS, and later extended their work to the purification of PVM using PEG of different molecular weights and concentrations (35% solution of PEG 4000, 30% solution of PEG 6000, and 20% solution of PEG 15000) (Albrechtová and Klír, 1970).

In PVM purification Hödrejärv et al. (1970) homogenized infected leaves of *S. demissum* in the presence of chloroform. The resulting clarified sap was subjected to differential centrifugation. Electrophoresis of the partially purified virus was then carried out in a sucrose density gradient column. Hiruki et al. (1974) used n-butanol and 2% (w/v) PEG 20000 without NaCl to purify PVM.

In PVS purification, Shepard (1970) clarified the virus-containing leaf homogenate with chloroform, subjected it to differential centrifugation and precipitated the virus with 5% (w/v) PEG 6000; the final purification was achieved by two additional cycles of differential centrifugation. Kozar and Sivers (1975) added 5% Triton X-100 to the phosphate buffer during homogenization of infected tomato leaves to increase the yield of PVS, which resulted in an 8-fold increase in serological titre of the virus preparation obtained.

Despite of the reports on the purifications of PVM and PVS in the past only a limited amount of information is available on the quantitative estimation of yield, specific infectivity and the physicochemical properties of both viruses. The purposes of the present study are: firstly, to purify these two viruses quantitatively by applying an improved PEG-NaCl precipitation technique and thus to provide large amounts of purified PVM and PVS for the later interaction studies; secondly, examination of the purity of the virus preparations by ultraviolet (UV) absorption, electron microscopy, analytical and buoyant density centrifugations and thirdly, determination of the specific infectivity of purified PVM and PVS by using their respective local lesion hosts.

## MATERIALS AND METHODS

### A. Virus

The Alberta isolate (AP-1) of PVM was propagated in 'King

Edward' potato. The infected plants were slightly stunted but otherwise appeared 'normal' (Plate 1, A). The Canadian 'isolate A' of PVS, free from infections with other viruses including potato viruses M, X, and Y (Hiruki, 1975a), was multiplied in 'Netted Gem' potato (Plate 1,A). In PVS-infected plants veinclearing and leafrolling symptoms were evident in the early stages of their growth. Freedom of the samples used in this study from PVX infection was ascertained by using *Gomphrena globosa* L. (Wilkinson and Blodgett, 1948). The potato plants were grown in 20-cm plastic pots containing a soil-mixture of 3 parts loam, 1 part peatmoss and 1 part coarse-particle-size sand, and fed with commercial fertilizer (28-14-14) at intervals of 2-4 weeks, depending on growth conditions. The infected potato leaves, excluding mid-ribs, were harvested just prior to the blossom stage (1.5-2.0 months), put in polyethylene bags (200gm/bag) and stored in a deep freezer at -63.5°C until use.

## B. Local Lesion Hosts

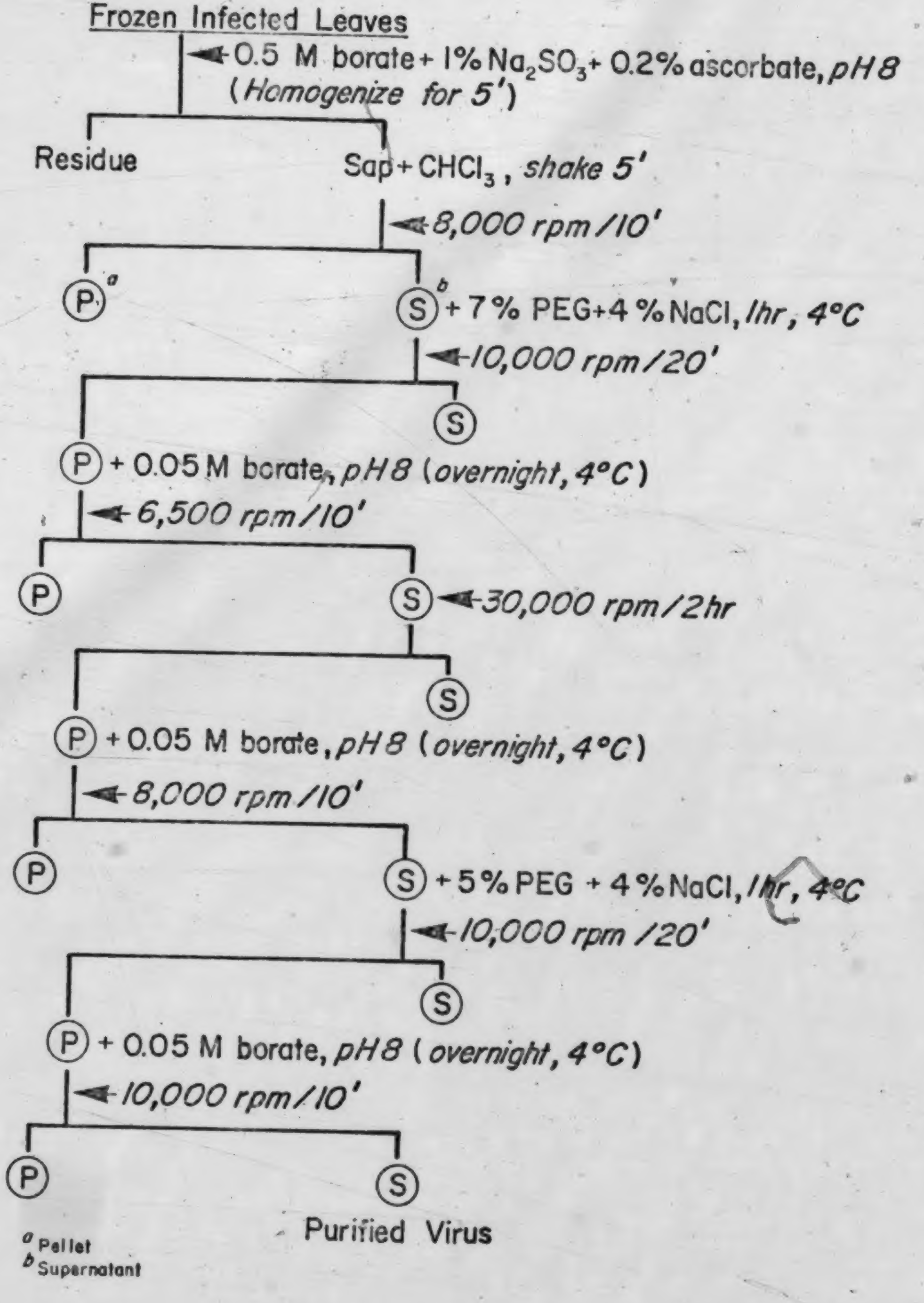
The selected seeds of 'Red Kidney' bean (RKB) (Town House brand, Empress Foods Ltd., Vancouver, B.C.) were sown in 10-cm clay pots containing steam-sterilized UC-mix II (C) (Matkin and Chandler, 1957). The plants were grown in a greenhouse at 17°±2°C and cool-white fluorescent tubes at an intensity of 5,380 lx (500 ft-c) (Hiruki et al., 1974) were used to maintain a 16-hr photoperiod per day during the winter. Only those seedlings with the trifoliate leaf-bud just emerged were selected for PVM bioassay (Hiruki, 1970). The RKB plants of this particular growth-stage were obtainable from 9 to 12 days after seeding, depending on season of the year (Hiruki, 1973).

*Chenopodium quinoa* Willd. seeds, the C-selection (Hiruki, 1975a), were initially germinated in disposable plastic trays (12 cm X 19 cm X 6 cm deep) containing UC-mix. The seeds were covered with a thin layer (3-4 mm) of the soil-mixture. After 10 to 12 days the uniform seedlings were selected and transplanted individually into the clay pots containing the soil-mixture. The plants were grown in the greenhouse and fertilized at regular intervals. The greenhouses were properly shaded on the roof throughout the summer while a 16-hr photoperiod per day was maintained with supplementary fluorescent and incandescent lights at an intensity of 16,000 lx in the winter. The *C. quinoa* plants were most susceptible to PVS during the period between 40-60 days after seeding (Hiruki, 1975a).

## C. Purification

The following method was used for purifying both PVM and PVS. Frozen infected-leaves (200 gm) were homogenized in the presence of 400 ml buffer solution, which contained 0.5 M borate, 1% (w/v) sodium sulphite and 0.2% (w/v) L-ascorbate, adjusted to pH 8.0 with sodium hydroxide (NaOH), for 5 min using a Waring Blendor (Fig. 1). The green-colored juice was squeezed through two layers of cheese-cloth into a beaker kept in ice. The resulting sap sample amounting to 550-600 ml was transferred to a 2-litre volumetric flask and shaken vigorously with an equal volume of chloroform (Schneider, 1953) for 5 min. The emulsified mixture was transferred to six 250-ml polypropylene bottles and centrifuged at 8,000 rpm for 10 min (Sorvall model RC 2-B refrigerated centrifuge, GSA rotor). After the centrifugation three distinct layers were obtained. They were an amber-colored supernatant containing the

FIG. I. A flow diagram illustrating the purification procedure of potato virus M and potato virus S.


Frozen Infected Leaves
0.5 M borate + 1% $Na_2SO_3$ + 0.2% ascorbate, pH 8
(Homogenize for 5')
Residue
Sap + $CHCl_3$, shake 5'
8,000 rpm/10'
P [a]
S [b] + 7% PEG + 4% NaCl, 1hr, 4°C
10,000 rpm/20'
S
P + 0.05 M borate, pH 8 (overnight, 4°C)
6,500 rpm/10'
P
S
30,000 rpm/2hr
S
P + 0.05 M borate, pH 8 (overnight, 4°C)
8,000 rpm/10'
P
S + 5% PEG + 4% NaCl, 1hr, 4°C
10,000 rpm/20'
S
P + 0.05 M borate, pH 8 (overnight, 4°C)
10,000 rpm/10'
P
S
Purified Virus
[a] Pellet
[b] Supernatant

virus, a whitish solid interphase coagulum (2.5-3.0 mm) of denatured protein and cell debris, and the chloroform solvent phase containing chloroplast-chlorophyll elements at the bottom. The supernatant was transferred into a 1-litre beaker kept in ice, to which a mixture of 7% (w/v) PEG 6000 (MW 6000-7500, J. T. Baker Chemical Co., N.J.) and 4% (w/v) NaCl was added with continuous stirring until dissolved. The solution was kept at 4°C for 1.0-1.5 hr (Wagner et al., 1970; Reddy and Black, 1973) to facilitiate virus precipitation before being centrifuged at 10,000 rpm for 20 min. The virus in a pelleted form was suspended in 150 ml 0.05 M borate buffer adjusted to pH 8.0 with NaOH (hereafter referred to as borate buffer), overnight at 4°C. The suspension was centrifuged at 6,500 rpm for 10 min and the resulting supernatant was further centrifuged at 30,000 rpm for 2 hr (Beckman model L-4 preparative ultracentrifuge, Spinco rotor 30). The pellets thus obtained consisted of virus as a clear gelatinous top-layer and the chloroplast debris at the bottom-layer. The top-layer was dissolved in 40 ml of borate buffer and kept overnight at 4°C. The virus suspension was centrifuged at 8,000 rpm for 10 min and the supernatant was reprecipitated with 5% PEG and 4% NaCl. The pellet was dissolved in 7-8 ml of borate buffer and kept overnight at 4°C. The supernatant containing the purified virus was finally obtained by centrifuging the suspension at 10,000 rpm for 10 min.

## D. Electron Microscopy

Droplets of diluted purified virus preparations were placed on 200-mesh Formvar-coated copper grids and a droplet of 2% neutral sodium phosphotungstate (PTA) was added to each sample (Brenner and Horne, 1959). A few seconds later the mixtures were blotted dry and the stained samples were examined immediately in a Philips 200 electron microscope at 60-80 kV. Leaf-dip preparations were also observed for comparison (Brandes, 1957).

## E. Analytical Centrifugation

The sedimentation coefficient values of the purified PVM and PVS preparations were determined by the sedimentation-velocity method performed in a Beckman model E analytical centrifuge with built-in high intensity UV optical system and monochromater assembly. The preparations of PVM or PVS purified by the PEG-NaCl method were subjected to further purification by 5-30% (w/v) sucrose density gradient centrifugation (Brakke, 1951) at 25,000 rpm (Spinco SW25.1 rotor) for 2.5 hr, prior to the examination by analytical centrifugation. The resulting gradient samples were fractionated by an ISCO model D density gradient fractionator, dialysed by 5-7 changes of 0.1 M phosphate buffer containing mono- and di-basic potassium phosphates, pH 7.0, and then centrifuged at 30,000 rpm at 20°C in the analytical centrifuge. The sedimenting boundary was monitored with UV optics at 2 or 4 min intervals for 24 min and the microdensitometer tracings of the films were obtained. In some runs the progression of the sedimenting boundary was measured by using a photoelectric scanner and a multiplexer accessory. The sedimentation coefficient was calculated by the graphical method (Markham, 1960) and was corrected to the solvent viscosity of water at 20°C.

## F. Buoyant Density

Solutions of 20, 30 and 40% (w/v) cesium chloride (CsCl) were made in 0.01 M Tris buffer containing 0.1 M NaCl and 1 mM ethylenediamine tetraacetate, pH 7.5. About 1 ml of the purified virus preparation was layered on the top of a discontinuous step gradient (Meselson et al., 1957) made by 0.5 ml 20%, 1.0 ml 30% and 1.5 ml 40% CsCl in a 5-ml lusteroid tube, then the gradient tubes were centrifuged at 35,000 rpm for 20 hr at 4°C (Beckman model L-2 ultracentrifuge, Spinco SW 50.1 rotor). Fractions of 0.2 ml each were obtained from the gradient by piercing the bottom of the centrifuge-tube and the absorbance of each fraction was determined at 260 nm. The refractive index of selected fractions was also obtained (Erma Refractometer, Tokyo) and converted to the density of CsCl in gm/ml (Anderson and Anderson, 1970). The virus fraction was dialysed against 8-10 changes of borate buffer and assayed for infectivity in its respective local lesion host.

## G. Standard Curves

PVM and PVS preparations purified by the PEG-NaCl method were further subjected to sucrose density gradient centrifugation (see Section F), dialysed against 10 changes of double distilled water, and were lyophilized in a freeze-dryer (Vir Tis Co., Inc., Gardiner, N.Y.) at -40°C for 4 days. A known weight of the lyophilized virus-preparation was dissolved in borate buffer to different concentrations for the determination of their absorbance (A) at 260 nm using a Hitachi Perkin-Elmer model 139 UV-VIS spectrophotometer (Fig. 2).

## H. Specific Infectivity

Specific infectivity was determined by inoculating a respective

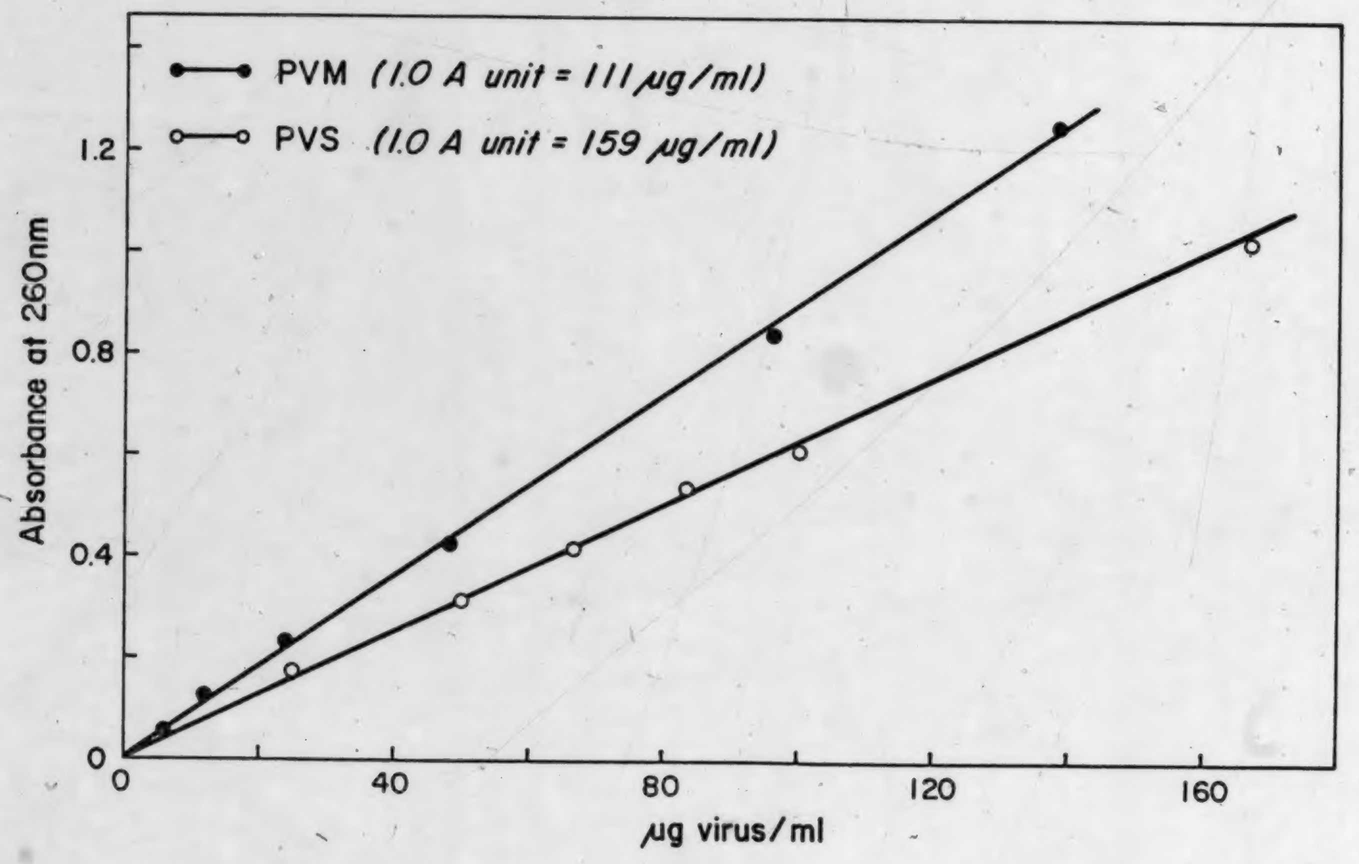


FIG. 2. Standard curves of purified potato virus M (PVM) and potato virus S (PVS): ultraviolet absorbance at 260 nm was used as a measure of virus concentration.

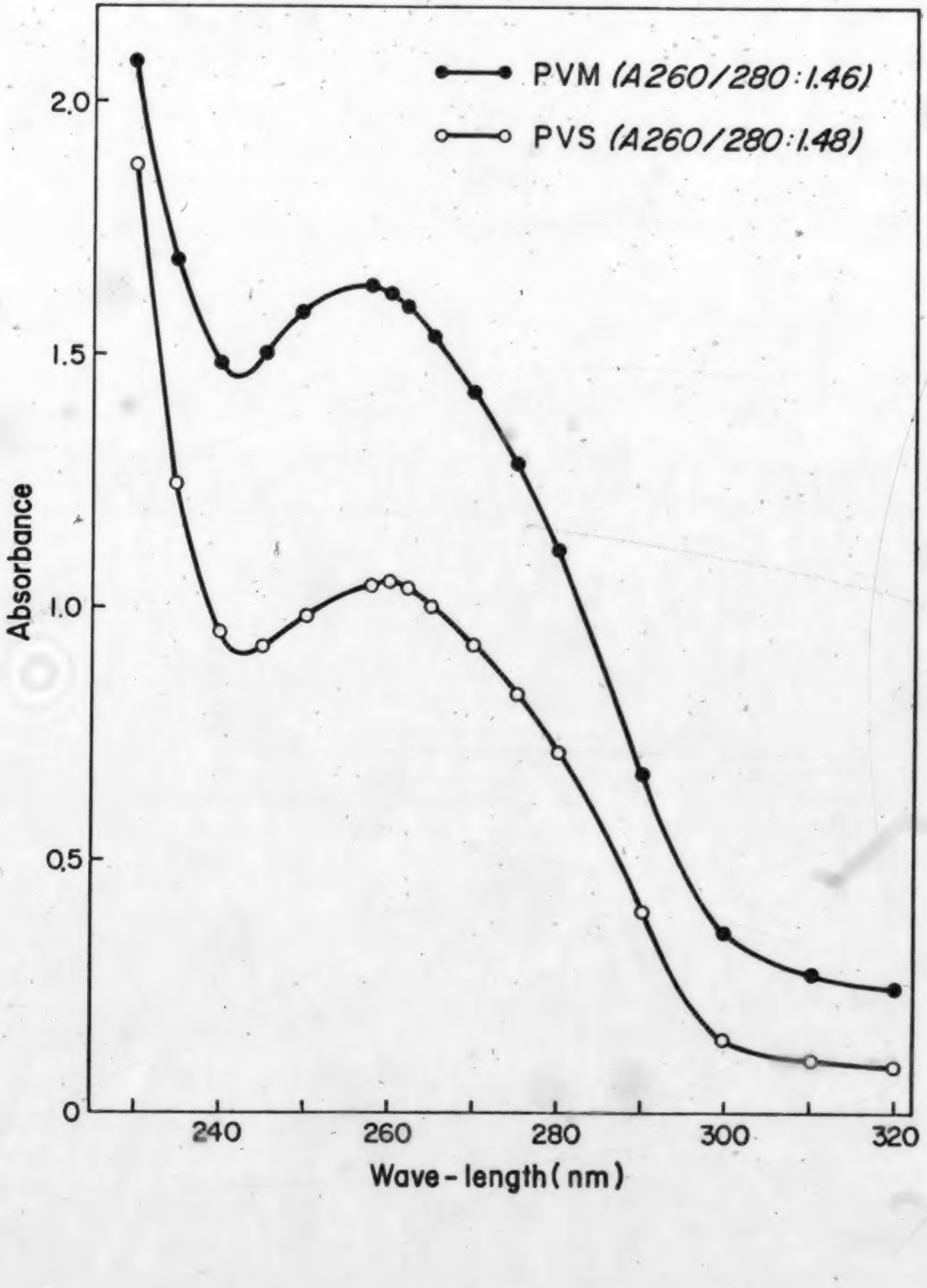
local lesion host with a known amount of purified PVM or PVS. The primary leaves of RKB were used for PVM assay, and before inoculation the buds of trifoliate leaves were removed. For PVS assay, 2-4 well-serrated leaves of each *C. quinoa* were selected for inoculation. The leaves were dusted uniformly with 600-mesh (20-22 µm) Carborundum (boron carbide, $B_4C$, Norton Co. Canada Ltd., Hamilton, Ontario) prior to inoculation. Inoculation was made by gently stroking the leaf surface with a Q-tip [Cheseborough-Pond's (Canada) Ltd., Toronto] dipped in inoculum. A piece of folded paper-towel of a convenient size was used to support the leaf at the under side during inoculation. Leaves were rinsed with distilled water immediately after inoculation. Two ml of virus suspension were applied separately to 62-64 half-leaves of RKB for PVM and 50-52 half-leaves of *C. quinoa* for PVS until the inoculum was exhausted.

The inoculated *C. quinoa* plants were incubated in growth chambers at 27°±2°C with a 16-hr photoperiod of 10,760 lx (1,000 ft-c) light intensity (Hiruki, 1975a), while RKB were incubated as described previously.

## RESULTS

Due to the combined strong anti-oxidizing effect of sodium sulphite (Bald and Samuel, 1934) and ascorbic acid (Best, 1939; Fulton, 1974) the extracted sap remained essentially green as expected. Purified preparations showed a UV-absorption spectrum, typical of purified virus preparations (Fig. 3). The values of A 260/280 were

FIG. 3. Ultraviolet-absorption spectra of purified potato virus M (PVM) and potato virus S (PVS) preparations.



1.46±0.04 and 1.48±0.03 for PVM and PVS respectively, which were within the range of the reported values for the carlavirus group (Table 1). The average virus yields were about 330 mg for PVM and 350 mg for PVS per kg of infected leaves.

Brown necrotic lesions (0.4-0.7 mm) (Plate 1, B) incited by PVM appeared in 3-4 days in RKB and were counted 8-9 days after inoculation. As to PVS, chlorotic lesions first appeared in 5 days, then increased in size (1.0-1.5 mm) with a necrotic centre in 7 days (Plate 1,C), and the maximum lesion number was obtained in 8-9 days after inoculation. The specific infectivities for purified PVM and PVS were 3 and 55 local lesions per $\mu$g virus respectively.

In electron microscopy the purified virus preparations were relatively free from host materials (Plate 2,A and B; Plate 3,A). However, in the length distribution of the virus preparations purified by the present method PVM showed three major peaks (Plate 2,C), while PVS formed a normal distribution pattern (Plate 3,B) with a modal length of 645-655nm (Wetter and Brandes, 1956; DeBokx, 1969; Hiruki and Shukla, 1973) when 300 particles were measured respectively.

The sedimentation coefficient ($s_{20,w}$) values, reported for the members of the carlavirus group, ranged from 136 to 172S (Table 1). In this investigation, the purified PVS preparation exhibited a single boundary in analytical centrifugation and the sedimentation coefficient was 148S. However, in the case of PVM, three sedimenting boundaries were noted, giving sedimentation coefficient values of 108, 99 and 90S respectively. This result seems to correlate with the histogram showing the particle length distribution of PVM (Plate 2,C), and may represent an artifact resulting from degradation of PVM during the prolonged

TABLE I. Comparison of some physical and biological properties of potato virus M (PVM), potato virus S (PVS) and other carlavirus members.

| | PVM | PVS | Carlaviruses[a] |
|---|---|---|---|
| Sedimentation Coefficient *($s_{20,w}$)* *(Svedbergs)* | 108(?) | 148 | 136-172 |
| Buoyant Density in CsCl *(gm/ml)* | 1.35 | 1.34 | 1.33[b] |
| A 260/280 | 1.46 | 1.48 | 1.09-1.55 |
| Yield *(mg virus/kg infected-leaves)* | 336 | 356 | 2-40[c] |
| Specific Infectivity *(Local lesion no./ μg virus)* of purified virus | 3 | 55 | — |

[a] Included are: carnation latent, chrysanthemum B, cowpea mild mottle, lily symptomless, narcissus latent virus, pea streak, red clover vein mosaic and poplar mosaic viruses (Source: C.M.I./A.A.B. Descriptions of Plant Viruses, 1970-1976).

[b] Narcissus latent virus (Brunt and Barton, 1976).

[c] Refer to the text for specific virus involved.

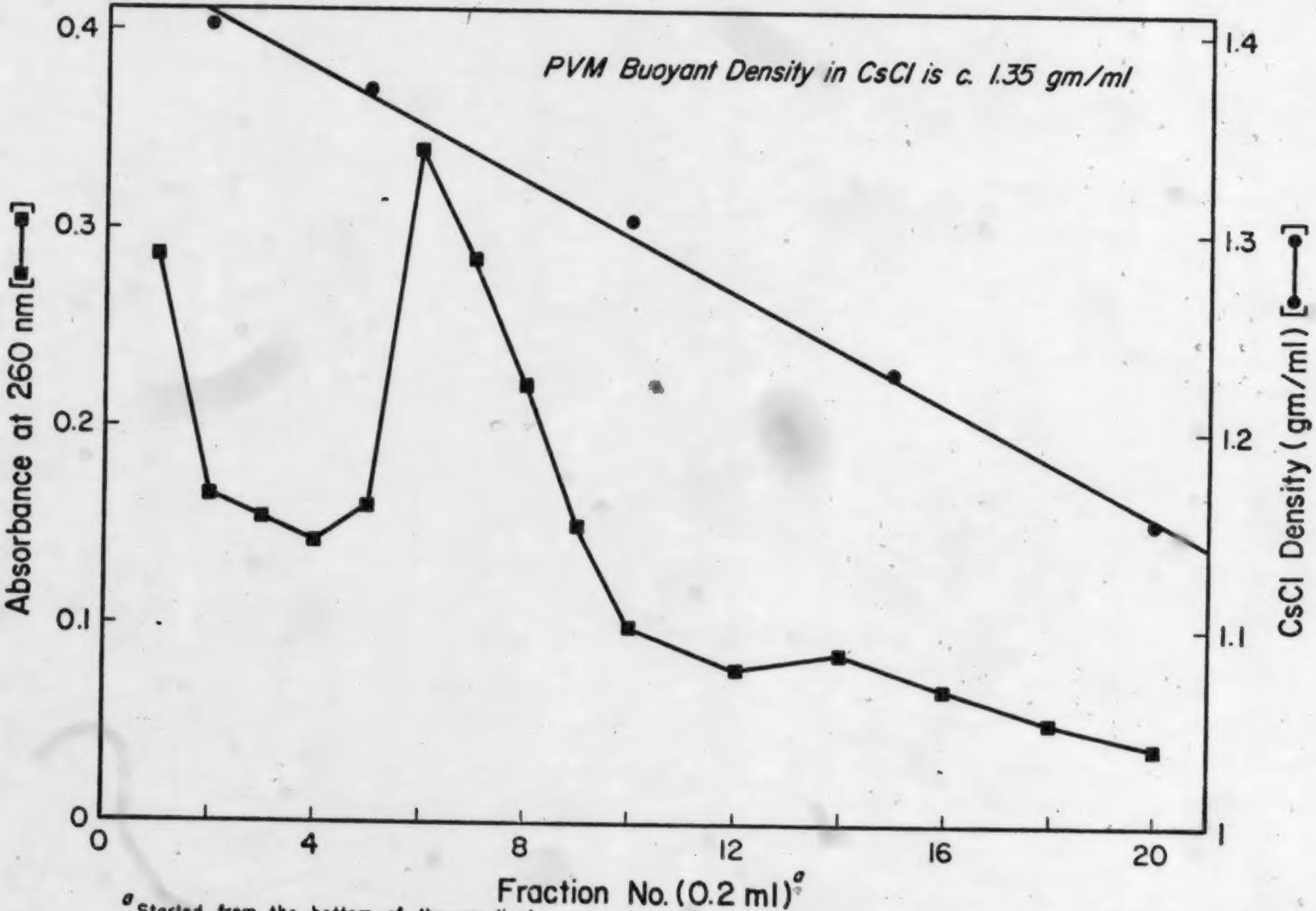
purification procedure used in this study. In equilibrium density gradient centrifugation, the virus preparations purified by the present method showed only a single light scattering band in the CsCl gradient (Plate 3,C) and a single UV-absorption peak (Figs. 4 and 5). The buoyant densities of PVM and PVS were found to be 1.35 and 1.34 gm/ml respectively (Figs. 4 and 5). The virus fraction recovered from the CsCl density gradient after exhaustive dialysis had extremely low infectivity for PVS (2.4 lesions/half-leaf in 24 half-leaves) and none for PVM when assayed on their respective local lesion hosts.

## DISCUSSION

A precipitation method using PEG and NaCl has been applied as a simple but efficient means of purifying bacteriophages, animal and plant viruses (Leberman, 1966; Gooding and Hebert, 1967; Kanarek and Tribe, 1967; van Kammen, 1967; Albrechtová and Klír, 1968; Yamamoto et al., 1970; Hsu and Black, 1973). Furthermore, the ease in the application of the procedures, another advantage of using this method, made quantitative purification of unstable viruses possible.

In separate experiments carried out along with this investigation, several other methods for PVM and PVS purification from the literature (see Introduction section) were tried out, but resulted in the virus yields ranging from 2 to 30 mg per kg of infected leaves (Lau, unpublished data). Hödrejärv et al. (1970) were able to obtain a yield of 10-15 mg purified PVM (strain $M_{11}$) per kg of leaf material. Other reported data on the yield of carlaviruses were: 2-5 mg for CMMV

FIG. 4. Buoyant density of potato virus M (PVM) determined by 20to40 % cesium chloride (CsCl) density gradient centrifugation.



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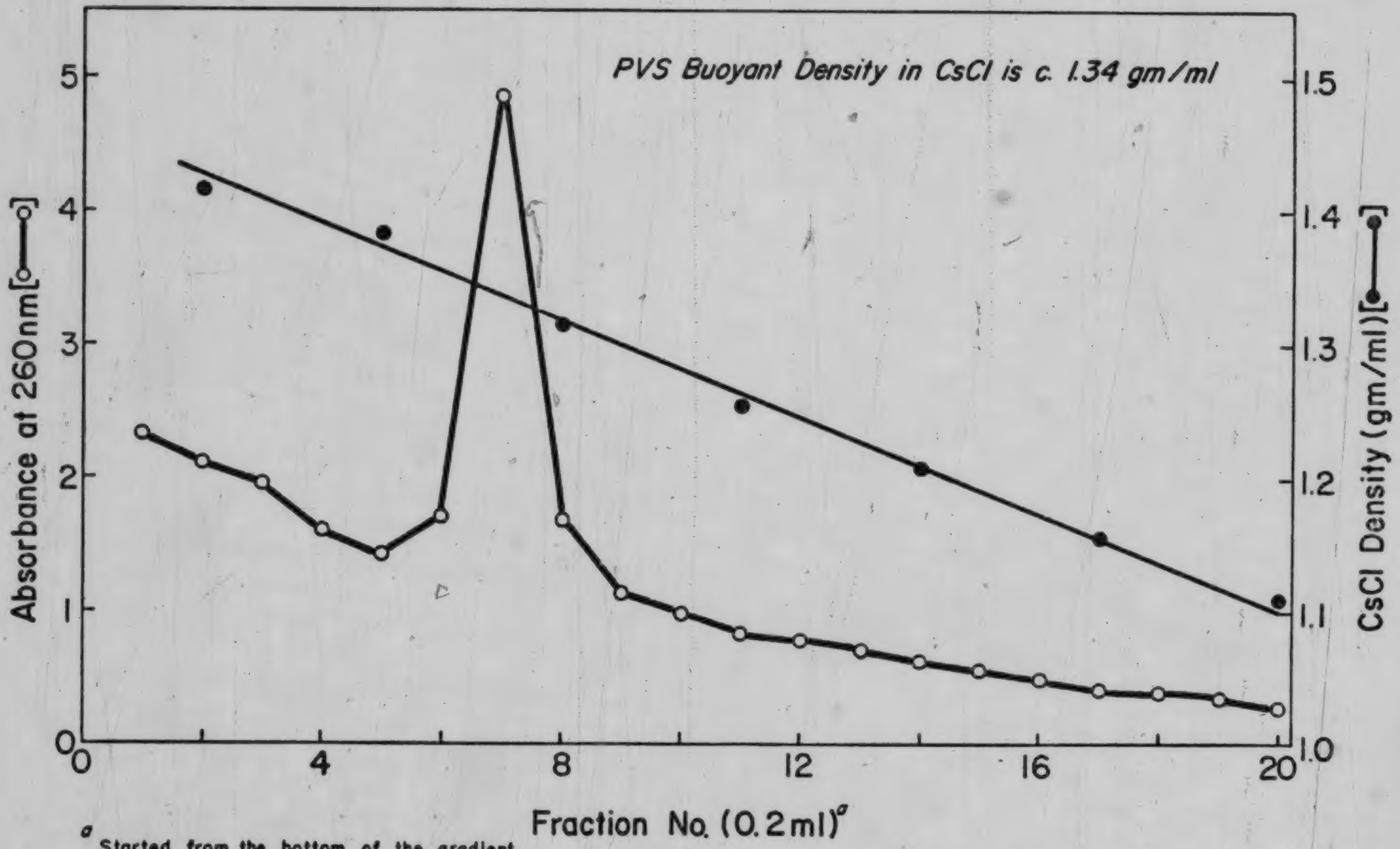
[a] Started from the bottom of the gradient.

FIG. 5. Buoyant density of potato virus S (PVS) determined by 20 to 40% cesium chloride (CsCl) density gradient centrifugation.



ª Started from the bottom of the gradient.

(Brunt and Kenten, 1973), c.25 and 40 mg per kg of infected leaves for NLV and PMV respectively (Brunt and Barton, 1976). All these authors did not use the PEG-NaCl method in their purification procedures. In the present method, however, about a 10-fold increase in yield of purified viruses over the previously reported values for PVM and PVS was obtained.

In this study, the brief mixing of chloroform with sap containing PVS in the initial step of purification did not result in virus inactivation as reported previously with other viruses (Damirdagh and Shepherd, 1970; Howell and Mink, 1976). However, the same treatment resulted in much lower specific infectivity in the case of PVM, which may indicate some partial degradation of virus particles as revealed by electron microscopy and sedimentation data.

Paliwal and Tremaine (1976) reported that side-to-side aggregation of ryegrass mosaic virus particles was minimized by suspending the virus in buffer at pH 8.0. More aggregation was found at acidic than at alkaline pH with CMMV, another carlavirus member (Brunt and Kenten, 1973). In the present investigation, the use of alkaline buffer was also found suitable in preventing virus aggregation. When the purified virus preparations were suspended in borate buffer and stored at -63.5°C, homogeneous virus suspensions were consistently obtained after thawing. In contrast, virus aggregates occurred after thawing the purified virus preparations that had been suspended in double distilled water (pH 5.6). Kaper and Siberg (1969) reported that turnip yellow mosaic virus (TYMV), suspended in water and incubated at -75°C, dissociated into RNA and capsid when thawed, and they suggested the RNA's bound to the insoluble protein fragments forming aggregates.

The values of the buoyant density of both viruses were similar and were comparable to that of the NLV (Table 1), the only other carlavirus member whose buoyant density value has been reported recently (Brunt and Barton, 1976). Yet the sedimentation coefficient of PVM was much lower than that of PVS and those of certain other carlavirus members. Besides degradation, perhaps many empty particles, especially those having the "normal length" might be present in the purified PVM preparations. However, the data pertaining to the UV-absorption spectrum of the PVM preparations used in this investigation did not suggest the presence of such defective forms to the extent detectable.

In contrast to isometric viruses (Bancroft, 1962), CsCl isopycnic banding resulted in tremendous losses of virus infectivity and thus was unsuitable for the purification of PVM and PVS. The exposure of other filamentous viruses to certain heavy salt solutions resulted in marked aggregation, and in some cases even complete degradation of virus particles occurred (Damirdagh and Shepherd, 1970; Rao, 1977). In this investigation, the UV-absorption spectrum of PVM indicated that a light-scattering material was present beneath the meniscus (fraction no. 14), indicating the occurrence of degradation of virus particles (Fig. 4).

Unlike ammonium sulphate, the precipitation with PEG-NaCl is more selective and the virus could be precipitated regardless of its concentration. Furthermore, if ribosomes were present initially in the preparation, they were not precipitated unless the concentration of PEG 6000 was increased to 10%(w/v) or higher (Yamamoto et al., 1970). Thus, the purified virus preparations by the present method were apparently free of ribosome contamination as indicated by electron microscopy (Plates 2,A and 3,A), analytical and buoyant density centrifugations. Shepard (1970) also reported a similar observation from the electron microscopy of the PEG-NaCl purified PVS and PVX preparations.

# CHAPTER IV

# BIOASSAY

## INTRODUCTION

Bagnall et al. (1956b, 1959) reported that PVM incited irregular reddish spot or ring-like lesions on the inoculated primary leaves of *Vigna sinensis* Endl. (cowpea) 14 days after inoculation, and that *D. metel* produced chlorotic local lesions 8 days after inoculation prior to systemic chlorotic spotting. As a local lesion host RKB is useful for PVM bioassay (Hiruki, 1970, 1973; Dziewońska and Ostrowska, 1973; Hiruki et al., 1974). Ross (1968) and Kowalska and Waś (1976) reported *L. chilense* Dun. as a reliable indicator plant for PVM.

With regard to assay hosts for PVS, Yarwood and Gold (1955) reported *Cyamopsis tetragonaloba* (L.) Taub. (guar) as a local lesion host. However, later it was found that these lesions were identical with those of PVM (Bagnall et al., 1956b, 1959). Ross (1968) also reported *L. chilense* as a useful assay plant for PVS. PVS incited necrotic lesions on *Saracha umbellata* Don. (Bagnall et al., 1956b) and *C. album* L. responded with chlorotic spots in about 20 days after inoculation (Bagnall et al., 1956b, 1959; Scholz, 1965b; Vulić and Hunnius, 1967b). Loughnane (1958) found that *Beta macrocarpa* (annual beet) developed local lesions in about 20 days. *C. amaranticolor* Coste and Reyn. was also found to be a local lesion host for PVS (Hollings, 1956), and Vulić and Hunnius (1967b) reported *C. quinoa, C. album, C. amaranticolor,* and *G. globosa* as the assay hosts for PVS in descending order of their sensitivity and efficiency. Other workers (Vulić and Hunnius, 1967b; de Bokx, 1970; Cupertino et al., 1970; Hiruki, 1975a; Kowalska and Waś, 1976) also agreed unanimously that *C. quinoa* was the useful local lesion host for PVS.

The optimal assay conditions for both local lesion host and virus inoculum have been studied extensively for PVM (Hiruki, 1970, 1973; Dziewońska and Ostrowska, 1973; Hiruki et al., 1974; Kowalska and Waś, 1976) and for PVS (Hiruki, 1975a; Kowalska and Waś, 1976) in respect to plant age, buffer concentration and pH, dilution curve, post-inoculation temperature and light intensity for maximal lesion development.

Environmental conditions, such as variations in illumination and temperature, influence the susceptibility of many host plants to virus infection. Bawden and Roberts (1947, 1948) discovered that pre-inoculation shading or darkening of test plants for 24 or 48 hr resulted from 2- to 10-fold increases in lesion number with 4 viruses tested. Subsequently, shading or darkening plants for 1-2 days before inoculation was employed routinely to increase the susceptibility of test plants with viruses that were difficult to transmit (Kassanis, 1949; Watson, 1952). At the same time, the significance of temperature effect on virus infectivity should not be overlooked. Post-inoculation temperature affected directly the local lesion production in assay hosts (Frost and Harrison, 1967; Hiruki, 1975a). Tobacco ringspot virus in sap was found inactivited after 1 day at 20°C, but retained its infectivity for 85 days at -5°C (Priode, 1928), while tomato spotted wilt virus was inactivated in 3 hr at room temperature (Bald and Samuel, 1934). Bawden and Pirie (1950, 1957) found that freezing minced leaves or expressed sap did not inactivate TNV.

In the present study, the effects of dark treatment and post-inoculation temperature on the local lesion hosts, and post storage temperature effect on virus infectivity were investigated. In addition, assays of PVM infectivity at different growth stages of infected potato plants and the reciprocal inoculations of PVM and PVS on their respective local lesion hosts were carried out.

## MATERIALS AND METHODS

Sap from infected potato leaves was extracted by using a mortar and pestle at a 1:10 (w/v) ratio in 0.057 M phosphate buffer containing mono- and di-basic potassium phosphates, pH 8.0 (hereafter referred to as phosphate buffer), as inoculum (Hiruki et al., 1974; Hiruki, 1975a) in all experiments described below. Inoculation procedure and incubation conditions were essentially the same as described before (Chapter III, Specific infectivity section). All experiments were done by using the half-leaf method with the balanced distribution of treatments wherever possible (Holmes, 1929; Samuel and Bald, 1933; Loring, 1937). Immediately after inoculation the leaf was tilted to one side for washing with distilled water from a wash-bottle to avoid contamination of the opposite half leaf.

## A. Dark Treatment

Five vigorously growing 8-day-old RKB and 5 uniformly grown 40-day-old *C. quinoa* (2 leaves/plant) plants were placed in a ventilated darkchamber [internal measurement (cm): 113(length), 60(width) and 45 (depth)] and kept in a greenhouse for 48-hr dark treatment at 20°±2°C. On the second day, the same number of assay plants from the same batches were added for 24-hr dark treatment. On the third day, the treated plants along with the same numbers of untreated plants from the same batches were inoculated with sap samples containing PVM and PVS respectively.

## B. Post-inoculation Temperature

RKB plants inoculated with PVM, 12 half-leaves per treatment, were placed separately in controlled growth chambers set at 17°, 22°, 27°, and 32°C, with a 16-hr photoperiod of 5,380 lx.

## C. Post Storage Temperature

### a. *Infectivity of PVM and PVS extracted from infected potato leaves after storage at different temperatures*

PVM-or PVS-infected potato leaves were harvested, cut, mixed, and divided into 3 batches of 0.2 gm each. The leaves were enclosed in polyethylene bags and one half of each virus sample was stored at 4°C for 6 days, while the remaining half was kept frozen at -63.5°C. After 3 days of storage at -63.5°C one of each virus sample was thawed at room temperature for 0.5 hr before being refrozen at -63.5°C. Infectivity of both viruses in the leaf samples from different temperature treatments was determined after 6-day-storage. Concurrently, fresh sap extracted from the leaves of the same PVM- or PVS-infected potato plants were tested as controls.

b. *Infectivity of PVM extracted from frozen infected potato leaves at various time intervals after incubation at 4° or at 24°C*

PVM-infected leaves were harvested from a 'King Edward' plant, cut, mixed, and divided into 11 batches of 0.2 gm each. Each sample was enclosed in a polyethylene bag and stored immediately at -63.5°C for 6 days before removing 5 batches each for incubation at 4° or at 24°C. For a control at zero time, infectivity assay was carried out immediately after extraction. Samples incubated at 4° or at 24°C were tested for their infectivity at 0.5-, 2-, 8-, 24- and 48-hr intervals.

## D. Assay of PVM Infectivity at Different Growth Stages of Infected Potato Plants

PVM-infected tubers of 'King Edward' were seeded as described before (Chapter II, Virus section). The first leaf samples, 0.2 gm each, were harvested from the first fully developed leaves 22 days after seeding. At the same time, the plant height and the height of leaves sampled were recorded. A total of 10 leaf samples was harvested at 3-day intervals, then individually enclosed in a polyethylene bag and stored immediately at -63.5°C until use.

## E. Reciprocal Inoculation

A total of 24 half-leaves of RKB and *C. quinoa* were inoculated respectively with a sap sample of PVM from 'King Edward' leaves. After 9 days, the inoculated leaves of *C. quinoa* were harvested and ground for back inoculation on RKB. Leaf-dip preparations from the inoculated

*C. quinoa* leaves were prepared by sliding the freshly cut leaf edge across a droplet of 2% neutral PTA resting on a 200-mesh Formvar-coated copper grid, blotted dry, and examined immediately in a Philips EM200 at 60-80 kV. Similar duplicated tests also were carried out for PVS in the reversal sequence of inoculation of the local lesion hosts.

## F. Infectivity Index

In the present study the following formula was used to obtain an infectivity index from local lesion counts.

$$\text{An infectivity index} = \frac{\text{Average number of local lesion obtained in test}}{\text{Average number of local lesion obtained in control}}$$

## RESULTS AND DISCUSSION

## A. Dark Treatment

The RKB plants that had been subjected to the dark treatment were pale in color and fragile upon touching. Two to 6-fold increases in susceptibility were demonstrated in local lesion number (Table 2). In both PVM and PVS infection the number of local lesions obtained was much higher in the 48-hr dark treatment. In an earlier experiment, where aluminum foil was used to cover the opposite half-leaf for dark treatment, no increase in susceptibility was observed (Hiruki et al., 1974). This might have been due in part to some possibilities that photosynthetic products as well as water are freely translocated throughout the plant so as to compensate the resulting effects of dark treatment in the covered portions of the leaves. Therefore, dark treatment of

TABLE 2. Effect of dark-treatment on the susceptibility of 'Red Kidney' bean (RKB) and *Chenopodium quinoa* to potato virus M and potato virus S, respectively.

| Local lesion host[a] | Plant no. | Control[b] | Dark-treatment[c] | |
|---|---|---|---|---|
| | | | 24 hr | 48 hr |
| R K B | 1 | 35 | 74 | 196 |
| | 2 | 28 | 35 | 153 |
| | 3 | 20 | 32 | 231 |
| | 4 | 32 | 51 | 94 |
| | 5 | 16 | 32 | 138 |
| Lesion no./half-leaf | | 6.6 | 11.2 | 40.6 |
| Standard error | | 0.9 | 2.0 | 5.9 |
| Infectivity index | | 1.0 | 1.7 | 6.2 |
| *C. quinoa* | 1 | 172 | 545 | 1256 |
| | 2 | 193 | 836 | 1101 |
| | 3 | 145 | 796 | 955 |
| | 4 | 322 | 1051 | 1613 |
| | 5 | 186 | 659 | 1321 |
| Lesion no./half-leaf | | 50.9 | 194.4 | 312.3 |
| Standard error | | 7.7 | 21.5 | 27.7 |
| Infectivity index | | 1.0 | 3.8 | 6.1 |

---

[a] Four half-leaves per plant were inoculated.

[b] Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

[c] Dark-chamber temperature: 20±2°C.

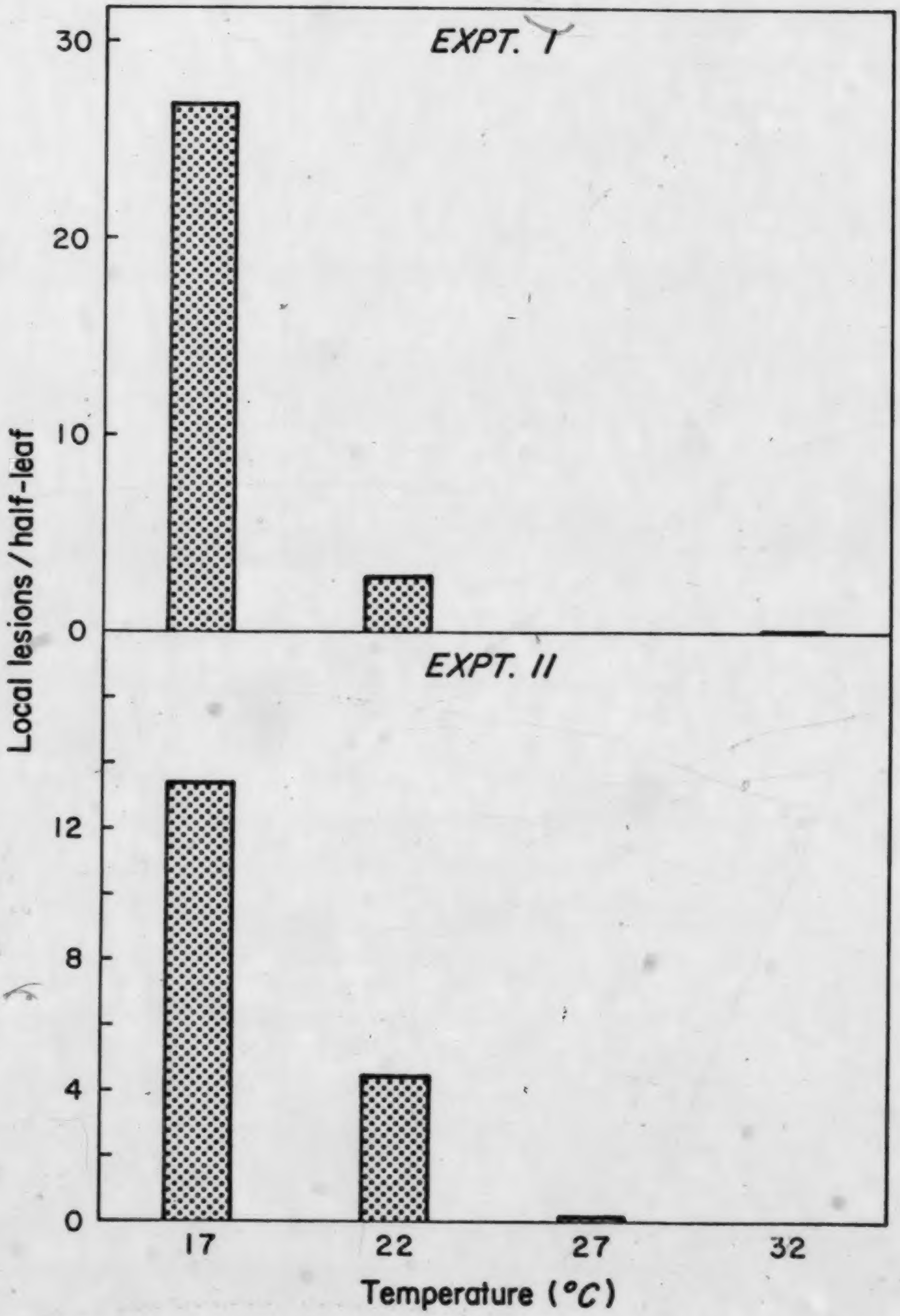
the whole plant is essential to obtain increased susceptibility. The results from the 24-hr dark treatment of *C. quinoa* essentially confirmed those of Hiruki's (1975a) although the 48-hr dark treatment in this investigation resulted in even higher susceptibility. It has been reported that the longer the test plants remained in the darkness (up to 6 days), the more susceptible they became to the subsequent virus infection (Bawden and Roberts, 1948; Costa and Bennett, 1955; Kimmins, 1967).

The inhibitory effect of photosynthetic products on virus multiplication was suggested initially (Bawden and Roberts, 1947, 1948; Yarwood, 1952; Wiltshire, 1956). However, when the inhibitory effect of untreated plant sap was tested against the sap from the dark-treated plant no significant difference was obtained (Bawden and Roberts, 1948). Kimmins (1967) and Kimmins and Litz (1967) found that pre-inoculation treatments in high humidity and darkness increased susceptibility, but when the relative humidity was reduced to 20%, the magnitude of the increase lessened in both darkened and illuminated plants. Therefore, these authors hypothesized that a greater number of infectible sites were rendered available in darkened plants by the increase of leaf turgor pressure.

## B. Post-inoculation Temperature

In two separate experiments RKB plants inoculated with PVM showed consistently much higher local lesion number at 17°C (Fig. 6), while at or over 27°C the number of lesions drastically decreased. Using the same local lesion host, Kowalska and Waś (1976) reported that in the temperature range between 16° to 22°C PVM lesions developed

FIG. 6. The effect of post-inoculation temperature on the local lesion number of potato virus M in 'Red Kidney' bean.



best, but not at 28°C. Using *D. metel* Bagnall et al. (1956b) found that the temperatures between 18° to 20°C were most suitable for the development of local and systemic spot symptoms incited by PVM. Even in potato plants symptoms of PVM infection was reportedly most pronounced at 16°C (Chrzanowska, 1973; Dziewońska and Ostrowska, 1973).

Post-inoculation temperature has its effects both directly on the virus and/or indirectly on the susceptibility of the host plant. Raising the temperature beyond the TIP of virus may increase the chances that particles become inactivated before they can start to reproduce in the host cells (Harrison, 1956). Frost and Harrison (1967) suggested that virus inactivating systems of the host were stimulated more efficiently at a higher temperature.

## C. Post Storage Temperature

### a. *Infectivity of PVM and PVS extracted from infected potato leaves after storage at different temperatures*

The highest infectivity was obtained from the constantly frozen leaf samples of PVM and PVS (Fig. 7). These results correlated well with Stanley's (1940) similar experiment in which at a 1:10 dilution of sap containing tomato bushy stunt virus a 1.6-fold increase in infectivity was obtained from the sample that had been frozen at -12°C over the fresh control sample. In the present virus-host systems, 1.3- to 1.4-fold increases in infectivity were obtained (Table 3). Harrison (1956) also found that the frozen sample of TNV-infected leaves gave higher infectivity than the fresh one or the sample that had been kept at 22° or at 30°C for 3 days. Therefore, freezing infected leaves, or sap from such leaves have little effect on some viruses (Matthews,

FIG. 7. Infectivity of potato virus M (PVM) and potato virus S (PVS), extracted from infected potato leaves, in 'Red Kidney' bean and *Chenopodium quinoa*, respectively, after storage at different temperatures. Infected fresh leaves from the same plants were used as controls.

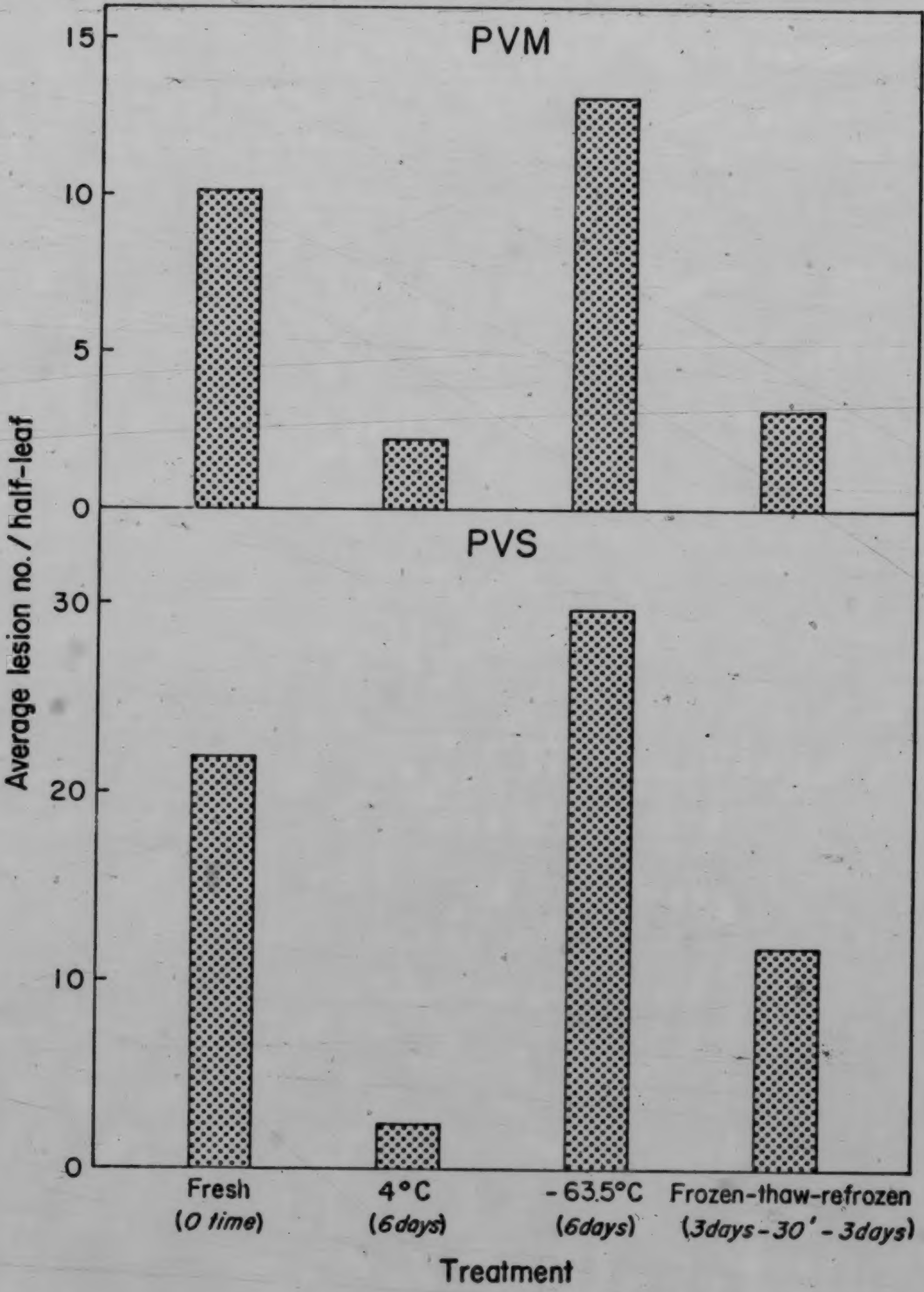


TABLE 3. Lesion counts: Infectivity of potato virus M and potato virus S, extracted from infected potato leaves, in 'Red Kidney' bean (RKB) and *Chenopodium quinoa*, respectively, after storage at different temperatures. Infected fresh leaves from the same plants were used as controls.

| Local lesion host[a] | Half-leaf no. | Fresh sap *(0 time)* | 4°C *(6 days)* | -63.5°C *(6 days)* | Frozen-thaw-refrozen *(3 days -30'-3 days)* |
|---|---|---|---|---|---|
| R K B | 1 | 9 | 2 | 12 | 2 |
| | 2 | 7 | 0 | 5 | 1 |
| | 3 | 17 | 6 | 20 | 6 |
| | 4 | 4 | 0 | 6 | 1 |
| | 5 | 5 | 1 | 6 | 1 |
| | 6 | 14 | 5 | 15 | 4 |
| | 7 | 10 | 2 | 19 | 5 |
| | 8 | 15 | 2 | 22 | 5 |
| | Mean | 10.1 | 2.3 | 13.1 | 3.1 |
| | Standard error | 1.7 | 0.8 | 2.4 | 0.7 |
| | Infectivity index | 1.0 | 0.2 | 1.3 | 0.3 |
| *C. quinoa* | 1 | 25 | 3 | 32 | 14 |
| | 2 | 14 | 0 | 10 | 6 |
| | 3 | 15 | 0 | 25 | 4 |
| | 4 | 34 | 4 | 45 | 20 |
| | 5 | 17 | 2 | 31 | 10 |
| | 6 | 19 | 2 | 32 | 12 |
| | 7 | 35 | 6 | 32 | 17 |
| | 8 | 16 | 2 | 31 | 11 |
| | Mean | 21.9 | 2.4 | 29.8 | 11.8 |
| | Standard error | 3.0 | 0.7 | 3.4 | 1.9 |
| | Infectivity index | 1.0 | 0.1 | 1.4 | 0.5 |

---

[a] Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

1970).

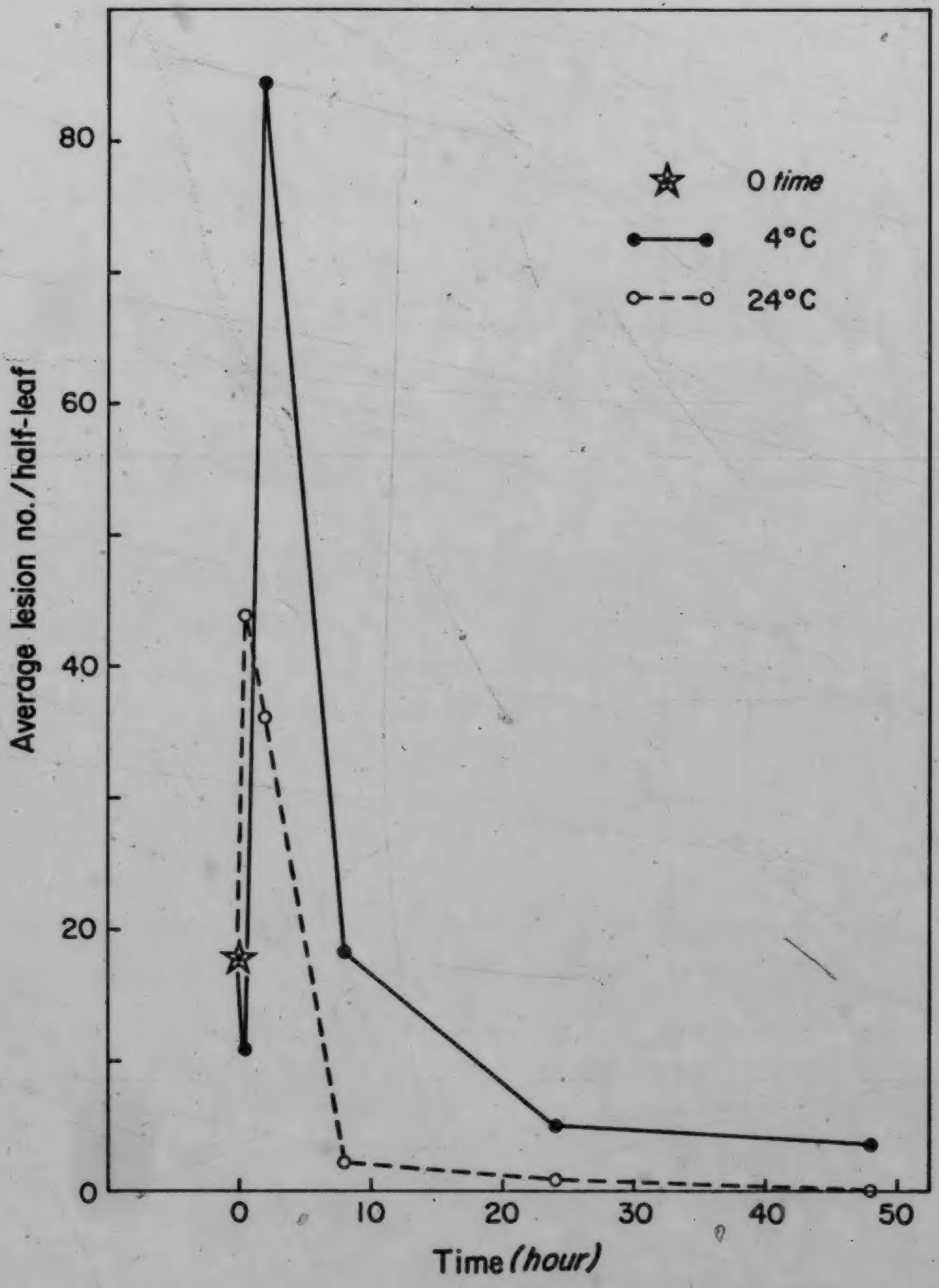
A plausible explanation for the higher infectivity obtained from the frozen samples could be that when the leaves thaw the virus particles are more readily released after the complete disorganization of the cells and that the oxidative enzymes are still in a less active state at such low temperatures. While temperatures near -20°C were claimed to be satisfactory for short term storage (c. 1 year) of some labile viruses (McKinney and Silber, 1968), Meryman (1956) found that all chemical denaturation processes would only appear to stop near -100°C.

The infectivity of the samples subjected to the frozen-thaw-refrozen treatment was lower than that of the fresh ones for both PVM and PVS (Fig. 7). However, the 30-min thawing of the frozen samples at room temperature was insufficient for virus inactivation.

### *b. Infectivity of PVM extracted from frozen infected potato leaves at various time intervals after incubation at 4° or at 24°C*

At zero time the local lesion number obtained was relatively low. PVM extracted from potato leaves that had been frozen and then incubated for 2 and 0.5 hr at 4° and at 24°C respectively, produced the highest number of local lesions (Fig. 8). Thereafter the lesion number steadily decreased up to 48 hr of incubation. Fulton (1957) reported that 4 *Prunus* virus isolates lost their infectivity rapidly in cucurbit sap at 24°C but this loss was slower at 0°C. Prune dwarf, sour cherry recurrent necrotic ringspot and necrotic ringspot viruses also were found losing their infecticity in sap after few hours at room temperature (Hampton and Fulton, 1961).

FIG. 8. Infectivity of potato virus M extracted from frozen infected potato leaves at various time intervals after their incubation at 4° or at 24° C.



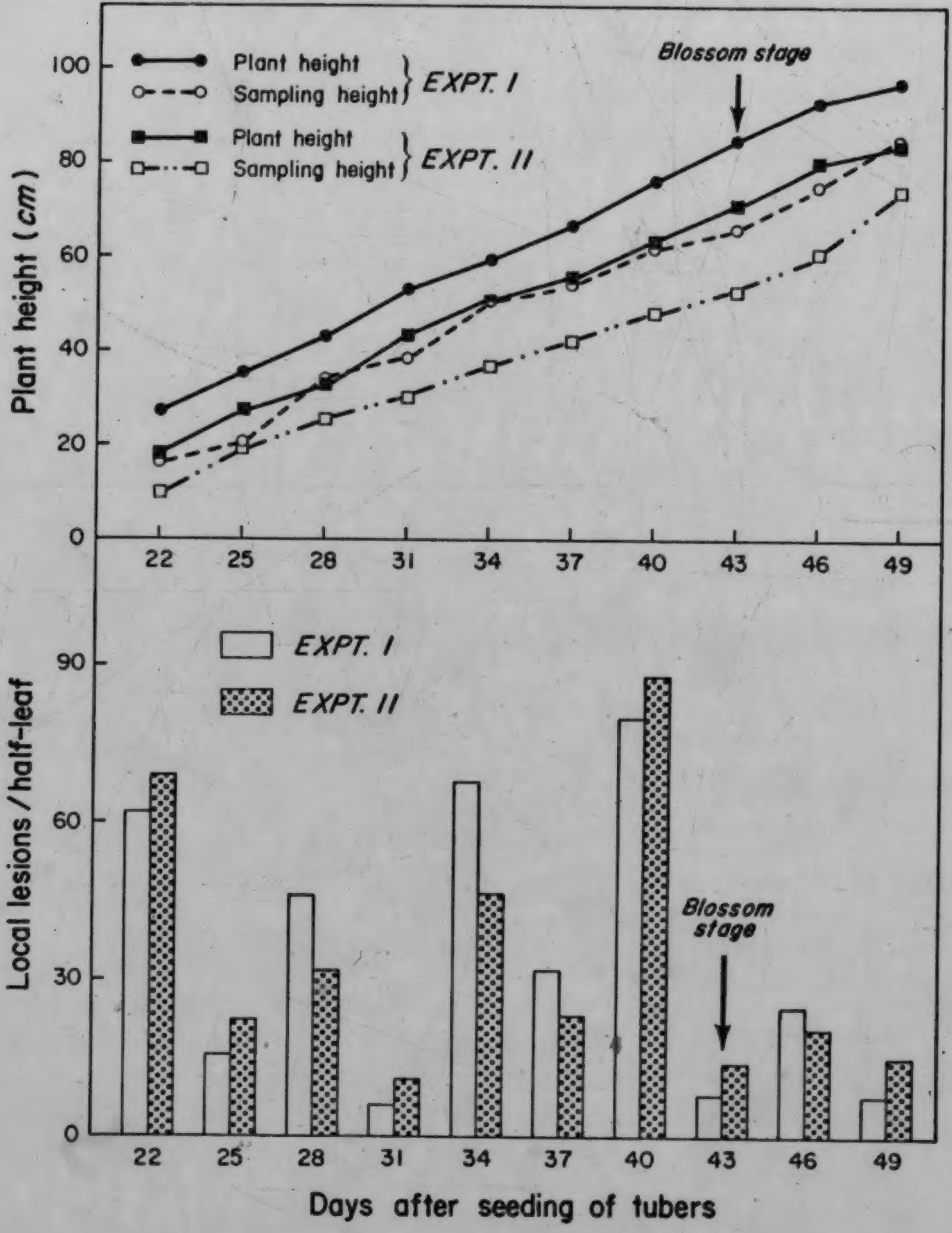
### D. Assay of PVM Infectivity at Different Growth Stages of Infected Potato Plants

In both experiments, the relative concentration of PVM estimated by bioassay was initially high, then fluctuated until it reached its maxima just before the blossom stage (Fig. 9). Virus concentration remained low after the appearance of blossoms. The concentration of PVM in infected 'King Edward' potato plants reached a maximum 40 days after seeding. This result agrees with Chrzanowska's (1973) in which serological detection of PVM was best with sap similarly obtained from the upper 2nd, 3rd and 4th leaves of plants aged 5 to 7 weeks. Bartels (1966) also found by serological tests that infected potato and tomato plants showed that PVM was evenly distributed throughout the foliage initially, but after 5 and 8 weeks, respectively, it was generally found only in the upper parts of the plants. Similarly, Wetter (1957) found that with most potato varieties tested, the PVS concentration was highest in leaves just below the growing tip of the shoots.

### E. Reciprocal Inoculation

PVM was not recovered from PVM-inoculated *C. quinoa* leaves to RKB; nor could PVM particles be demonstrated by electron microscopy using the same inoculated samples. Similarly, PVS was not detected from the RKB inoculated with PVS by using the same methods. These results agreed with earlier observations (Hiruki, 1970; Hiruki, unpublished data). Thus, taking advantage of this system the interaction of these two viruses could be studied in their respective local lesion hosts.

FIG. 9. Determination of the infectivity of potato virus M in 'Red Kidney' bean at different growth stages of infected 'King Edward' potato plants.



# CHAPTER V

# INTERACTION BETWEEN PVM AND PVS IN LOCAL LESION HOST

## INTRODUCTION

Although PVM and PVS coexisted in 'King Edward' potatoes, their intermediate types were never found, and this result suggested the lack of genetic recombination between them (Kassanis, 1963). The biological interaction between these two viruses, nevertheless, still remain to be understood. As seen in the foregoing literature review, the use of sap as virus inoculum in virus interaction studies may inevitably result in some undesirable interference between host inhibitors and the interacting viruses, leading to unclear, inconclusive results. Therefore, in the present study purified preparations of PVM, PVS, and their RNA and protein components were used to investigate their biological interactions quantitatively in their respective local lesion hosts.

## MATERIALS AND METHODS

### A. Interaction Between Intact PVM and PVS

### a. *Simultaneous inoculation*

Purified PVM and PVS were mixed in ratios of 1:1, 1:5 and 1:10 respectively, and the volume of each mixed-virus inoculum was kept constant. The inocula containing PVM and PVS, paired with the inoculum containing PVM only as a control, were applied to RKB. The same treatments, except in the reverse order, were also carried out in *C. quinoa.*

### b. *Pre- and post-inoculations*

In pre-inoculation treatments, inoculation with PVM was carried out using 12 half-leaves of RKB per treatment, and was followed by the secondary inoculations with PVS at 0-, 1-, 5-, and 10-hr intervals respectively. In post-inoculation treatments, inoculation in the reverse order was made also using RKB at the same time. Inoculum containing PVM and phosphate buffer, replacing the same volume of PVS, was applied as a control on the opposite half-leaves. Phosphate buffer alone was also applied to the control half-leaves at the same time when the second virus was applied at the scheduled time intervals. Daily counts of local lesions were made at the fixed time as soon as they were visible. The increment curves were obtained from the local lesion counts for each treatment. The same procedures were used in assaying the infectivity of PVS in the presence of PVM in *C. quinoa.*

### c. *Simultaneous separate inoculations of upper and lower epidermal layers*

Inoculation with PVM was made to the upper epidermis of 12 whole leaves from 12 RKB plants. The lower epidermis of half-leaves was inoculated with PVS and the remaining half-leaves with phosphate buffer. Inoculations were made in the reverse order to the other 12 leaves.

Similar treatments, in the reverse sequence, were repeated in *C. quinoa*.

## B. Interaction Between Intact Virus, Viral Ribonucleic Acids (RNA's), Yeast-RNA and Coat-protein

### a. *Isolation of viral RNA's and coat-proteins*

The viral RNA's were isolated by the phenol method (Fraenkel-Conrat et al., 1961). The UV-absorption spectrum and the infectivity of each viral RNA preparation were determined. The coat-proteins were isolated by the acetic acid method (Fraenkel-Conrat, 1957) and the resulting preparations were free from intact virus particles when subjected to electron microscopic examinations. The coat-proteins were dissolved in 0.1 N sodium hydroxide, adjusted to pH 8.0, and stored in a deep freezer until use.

### b. *Interaction Between intact virus and viral RNA of the counterpart*

A fixed amount of PVM was added to two concentrations of PVS-RNA and applied separately to RKB plants. The same concentration of PVM was used as a control. Inoculum containing PVS and PVM-RNA was similarly tested in *C. quinoa*, with a comparable control containing PVS only.

### c. *Simultaneous inoculation with PVM- and PVS-RNA's*

A fixed amount of PVM-RNA was mixed separately with two samples containing different amounts of PVS-RNA for bioassay in RKB, using PVM-RNA only as a control on the opposite half-leaf. Similar treatments were also carried out in the reverse order in *C. quinoa*.

### d. *Simultaneous inoculation with intact virus and viral coat-protein of the counterpart*

A fixed amount of PVM was mixed with two preparations containing different amounts of PVS coat-protein and the mixtures were applied separately to RKB plants. Inoculum containing PVM only was applied as a control. The same procedures were applied to *C. quinoa* using inocula containing PVS and PVM coat-protein as tests and PVS only as a control.

### e. *Detection of endogenous ribonuclease (RNase) activity*

A fixed amount of PVM-RNA was mixed with a series of 10-fold dilutions of PVS using phosphate buffer. Five inocula containing PVM and PVS in different ratios were prepared and were applied separately to RKB plants. Unmixed PVM-RNA inoculum was used as a control. The same treatments were carried out in *C. quinoa* with PVS-RNA plus PVM as test inoculum and PVS-RNA as a control.

### f. *Effect of endogenous RNase on PVS-RNA infectivity in the presence of yeast-RNA*

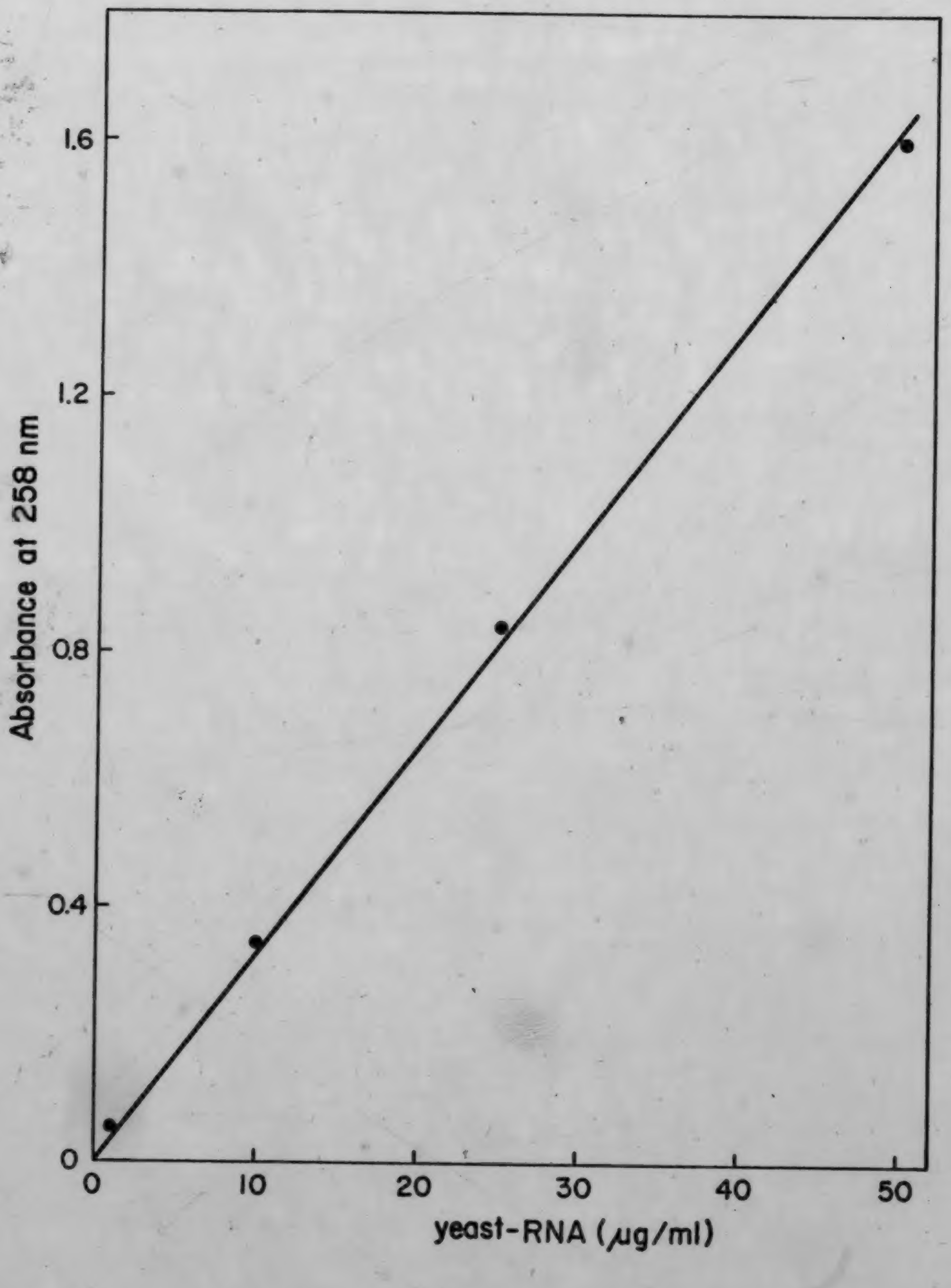
A standard concentration curve of yeast-RNA (Calbiochem, Los Angeles) was obtained by determining absorbance of solutions containing different amount of yeast RNA at 258 nm (Fig. 10). Fixed amounts of PVS-RNA and PVM were mixed with phosphate buffer and two different concentrations of yeast-RNA respectively. The three mixed-inocula were applied separately to *C. quinoa*, while PVS-RNA at the same concentration was applied to the opposite half-leaf as a control. The experiment was repeated with a higher concentration of PVS-RNA.

### g. *Stimulatory index*

In the present study the following formula was used to obtain a stimulatory index from local lesion counts.

$$\text{A stimulatory index} = \frac{\text{Average number of local lesion obtained in test}}{\text{Average number of local lesion obtained in control}}$$

FIG. 10. A Standard curve showing the absorbance values of yeast-ribonucleic acid (RNA) of different concentrations determined at 258 nm.



# RESULTS

## A. Interaction Between Intact PVM and PVS

### *a. Simultaneous inoculation*

Marked increases in local lesion number were obtained in RKB and *C. quinoa* in the presence of the counterpart (Figs. 11 and 12). In RKB the increase was more prominent as the amount of added PVS increased and the stimulatory index ranged from 1.4 to 7.1 (Tables 4A and 4B). However, in *C. quinoa* the lesion numbers incited by inocula containing PVM and PVS in the ratios of 1:5 and 1:10 did not differ significantly. The stimulatory index ranged from 1.4 to 2.1 for PVS in *C. quinoa* (Tables 5A and 5B). In application of phosphate buffer only, no lesion was incited in both hosts as expected.

### *b. Pre- and post-inoculations*

At zero time immediate, sequential inoculations were made with PVM and PVS separately. Similar inoculations in the reverse order were made as well. These treatments consistently resulted in the highest infectivity of the test virus, and the extent of the stimulatory effect was comparable to that obtained from simultaneous inoculation experiments described in the foregoing section. The pre or post-inoculations with PVM or PVS at different time intervals resulted in the limited stimulation of the infectivity of the counterpart (Figs. 13A and 13B). In general, the post-inoculation experiments showed slightly higher stimulatory indexes than those obtained in the pre-inoculation experiments in both hosts (Table 6.). The stimulatory indexes decreased as the time interval between the pre- and post-inoculations was prolonged.

FIG. 11. Increases in the local lesion number of potato virus M obtained by applying a mixed inoculum of purified potato viruses M and S in different ratios to 'Red Kidney' bean.

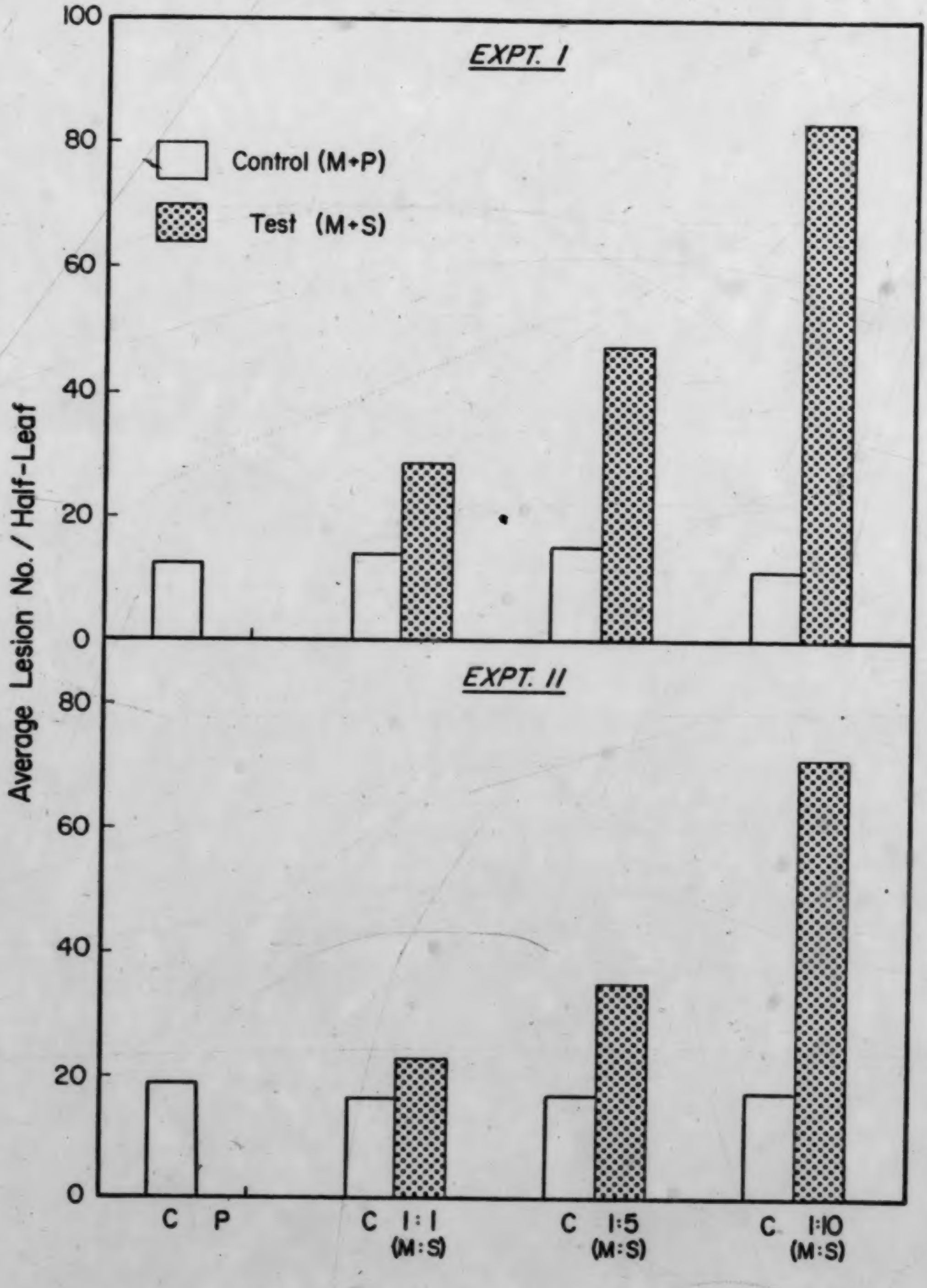


FIG. 12. Increases in the local lesion number of potato virus S obtained by applying a mixed inoculum of purified potato viruses S and M in different ratios to *Chenopodium quinoa.*

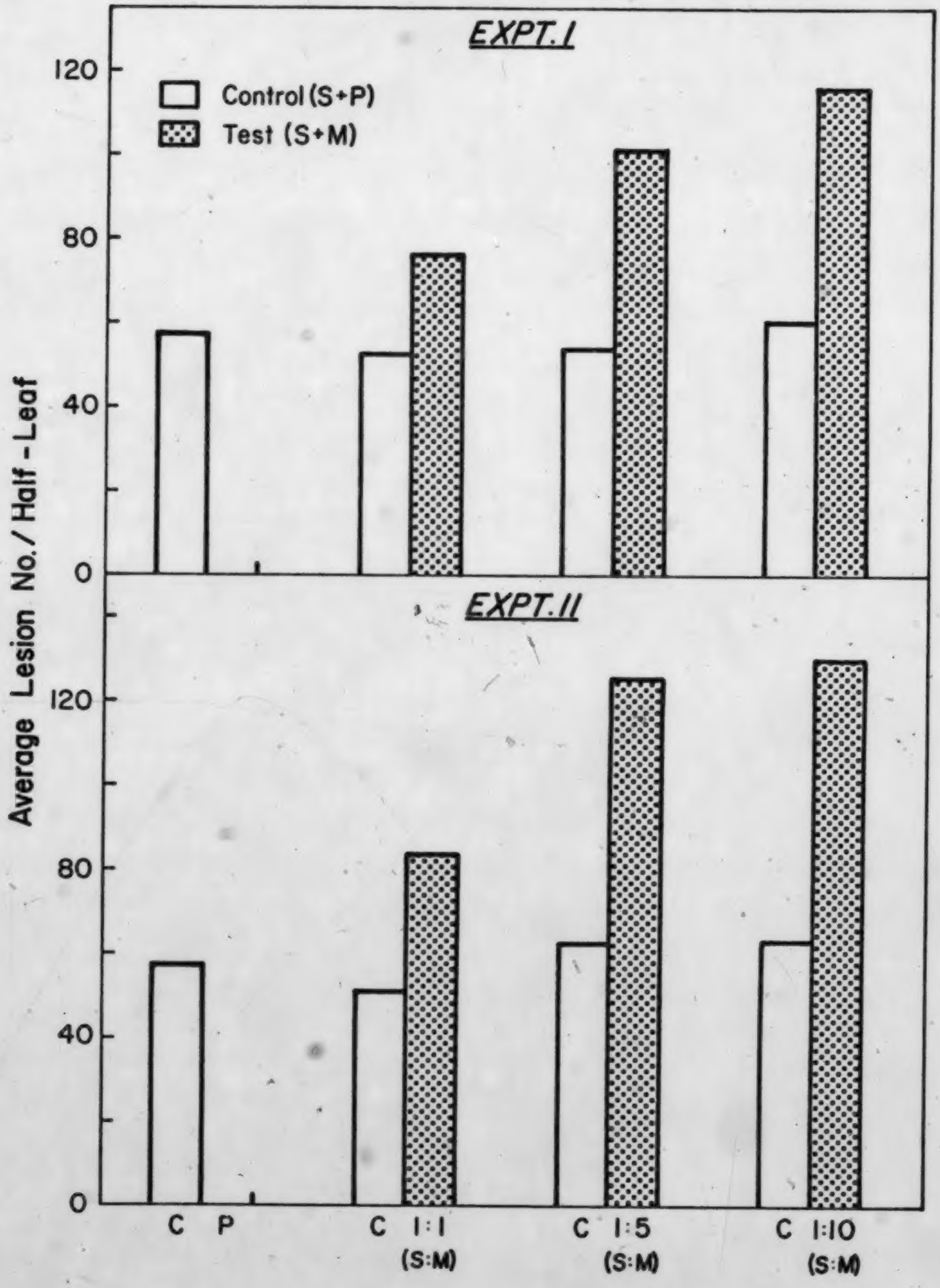


TABLE 4 A. The local lesion numbers obtained from simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) mixed in different ratios to 'Red Kidney' bean. *Expt. I.*

| Half-leaf no. | Lesion no./half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C[a] | P[b] | C | 1:1[c] | C | 1:5[c] | C | 1:10[c] |
| 1 | 8 | 0 | 12 | 20 | 20 | 39 | 12 | 81 |
| 2 | 13 | 0 | 21 | 19 | 17 | 30 | 7 | 54 |
| 3 | 22 | 0 | 6 | 24 | 15 | 33 | 23 | 96 |
| 4 | 11 | 0 | 11 | 30 | 13 | 48 | 16 | 67 |
| 5 | 5 | 0 | 13 | 32 | 12 | 23 | 10 | 45 |
| 6 | 13 | 0 | 14 | 17 | 21 | 28 | 6 | 73 |
| 7 | 11 | 0 | 7 | 36 | 19 | 79 | 7 | 64 |
| 8 | 7 | 0 | 10 | 50 | 6 | 59 | 11 | 85 |
| 9 | 12 | 0 | 21 | 15 | 28 | 75 | 9 | 133 |
| 10 | 6 | 0 | 26 | 11 | 12 | 57 | 13 | 101 |
| 11 | 12 | 0 | 12 | 54 | 9 | 21 | 25 | 110 |
| 12 | 15 | 0 | 24 | 38 | 11 | 57 | 12 | 96 |
| 13 | 27 | 0 | 9 | 23 | 13 | 64 | 8 | 69 |
| 14 | 10 | 0 | 11 | 32 | 20 | 35 | 5 | 97 |
| Mean | 12.3 | 0 | 14.1 | 28.6 | 15.4 | 46.3 | 11.7 | 83.6 |
| Standard error | 1.6 | 0 | 1.7 | 3.4 | 1.5 | 5.1 | 1.6 | 6.3 |
| Stimulatory index | | | | 2.0 | | 3.1 | | 7.1 |

[a] C = control, PVM only.

[b] P = 0.057 M phosphate buffer (pH 8.0) only.

[c] 1:1 = 1 part PVM + 1 part PVS ; 1:5 = 1 part PVM + 5 parts PVS ; 1:10 = 1 part PVM + 10 parts PVS.

TABLE 4B. The local lesion numbers obtained from simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) mixed in different ratios to 'Red Kidney' bean. *Expt. II.*

| Half-leaf no. | Lesion no. / half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C[a] | P[b] | C | 1:1[c] | C | 1:5[c] | C | 1:10[c] |
| 1 | 16 | 0 | 13 | 11 | 12 | 24 | 13 | 97 |
| 2 | 8 | 0 | 20 | 12 | 9 | 31 | 32 | 128 |
| 3 | 30 | 0 | 12 | 17 | 27 | 48 | 25 | 101 |
| 4 | 23 | 0 | 12 | 29 | 17 | 39 | 25 | 125 |
| 5 | 16 | 0 | 18 | 33 | 13 | 34 | 14 | 78 |
| 6 | 16 | 0 | 25 | 24 | 8 | 25 | 22 | 78 |
| 7 | 23 | 0 | 14 | 15 | 21 | 36 | 8 | 61 |
| 8 | 13 | 0 | 10 | 11 | 11 | 41 | 17 | 45 |
| 9 | 29 | 0 | 16 | 34 | 19 | 43 | 15 | 39 |
| 10 | 10 | 0 | 12 | 57 | 22 | 32 | 11 | 31 |
| 11 | 13 | 0 | 17 | 12 | 35 | 44 | 9 | 35 |
| 12 | 11 | 0 | 27 | 17 | 24 | 32 | 15 | 49 |
| 13 | 18 | 0 | 22 | 23 | 22 | 24 | 18 | 44 |
| 14 | 14 | 0 | 14 | 11 | 16 | 41 | 21 | 89 |
| 15 | 19 | 0 | 10 | 39 | 9 | 34 | 14 | 98 |
| 16 | 31 | 0 | 13 | 34 | 12 | 48 | 10 | 52 |
| 17 | 18 | 0 | 15 | 37 | 13 | 10 | 30 | 79 |
| 18 | 27 | 0 | 15 | 13 | 10 | 18 | 23 | 78 |
| 19 | 13 | 0 | 21 | 19 | 25 | 37 | 17 | 100 |
| 20 | 19 | 0 | 16 | 17 | 12 | 23 | 16 | 54 |
| 21 | 15 | 0 | 21 | 19 | 23 | 37 | 24 | 75 |
| 22 | 21 | 0 | 13 | 26 | 14 | 14 | 10 | 68 |
| 23 | 33 | 0 | 20 | 17 | 23 | 45 | 12 | 54 |
| 24 | 21 | 0 | 16 | 27 | 12 | 76 | 16 | 52 |
| Mean | 19.0 | 0 | 16.3 | 23.1 | 17.0 | 34.8 | 17.4 | 71.3 |
| Standard error | 1.4 | 0 | 0.9 | 2.3 | 1.4 | 2.8 | 1.3 | 5.6 |
| Stimulatory index | | | 1.4 | | 2.1 | | 4.1 | |

[a] C = control, PVM only.

[b] P = 0.057 M phosphate buffer (pH 8.0) only.

[c] 1:1 = 1 part PVM + 1 part PVS ; 1:5 = 1 part PVM + 5 parts PVS ; 1:10 = 1 part PVM + 10 parts PVS.

TABLE 5A. The local lesion numbers obtained from simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) mixed in different ratios to *Chenopodium quinoa*. *Expt. I.*

| Half-leaf no. | Lesion no. / half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C[a] | P[b] | C | 1:1[c] | C | 1:5[c] | C | 1:10[c] |
| 1 | 44 | 0 | 59 | 71 | 42 | 109 | 58 | 111 |
| 2 | 63 | 0 | 47 | 57 | 66 | 131 | 47 | 118 |
| 3 | 53 | 0 | 39 | 52 | 40 | 83 | 63 | 140 |
| 4 | 75 | 0 | 31 | 53 | 33 | 99 | 53 | 103 |
| 5 | 29 | 0 | 30 | 48 | 39 | 64 | 47 | 109 |
| 6 | 39 | 0 | 27 | 44 | 41 | 82 | 68 | 129 |
| 7 | 45 | 0 | 31 | 67 | 50 | 83 | 84 | 134 |
| 8 | 68 | 0 | 53 | 86 | 46 | 97 | 104 | 174 |
| 9 | 31 | 0 | 83 | 102 | 22 | 74 | 37 | 70 |
| 10 | 44 | 0 | 119 | 142 | 27 | 89 | 47 | 87 |
| 11 | 69 | 0 | 86 | 117 | 40 | 106 | 70 | 143 |
| 12 | 94 | 0 | 73 | 124 | 30 | 138 | 90 | 161 |
| 13 | 40 | 0 | 41 | 67 | 40 | 67 | 30 | 75 |
| 14 | 54 | 0 | 58 | 89 | 43 | 71 | 41 | 85 |
| 15 | 83 | 0 | 45 | 64 | 91 | 118 | 53 | 74 |
| 16 | 59 | 0 | 32 | 53 | 66 | 92 | 50 | 87 |
| 17 | 33 | 0 | 44 | 61 | 46 | 72 | 40 | 74 |
| 18 | 67 | 0 | 75 | 119 | 76 | 95 | 38 | 57 |
| 19 | 78 | 0 | 80 | 106 | 75 | 86 | 58 | 96 |
| 20 | 104 | 0 | 73 | 93 | 83 | 109 | 66 | 103 |
| 21 | 37 | 0 | 30 | 47 | 62 | 111 | 68 | 119 |
| 22 | 57 | 0 | 33 | 35 | 78 | 138 | 51 | 144 |
| 23 | 50 | 0 | 36 | 56 | 93 | 150 | 90 | 171 |
| 24 | 73 | 0 | 43 | 76 | 69 | 176 | 106 | 219 |
| Mean | 57.9 | 0 | 52.8 | 76.2 | 54.1 | 101.7 | 60.8 | 116.0 |
| Standard error | 4.1 | 0 | 4.8 | 6.0 | 4.3 | 5.8 | 4.3 | 8.1 |
| Stimulatory index | | | | 1.4 | | 1.9 | | 1.9 |

[a] C = control, PVM only.

[b] P = 0.057 M phosphate buffer (pH 8.0) only.

[c] 1:1 = 1 part PVM + 1 part PVS ; 1:5 = 1 part PVM + 5 parts PVS; 1:10 = 1 part PVM + 10 parts PVS.

TABLE 5B. The local lesion numbers obtained from simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) mixed in different ratios to *Chenopodium quinoa*. *Expt. II.*

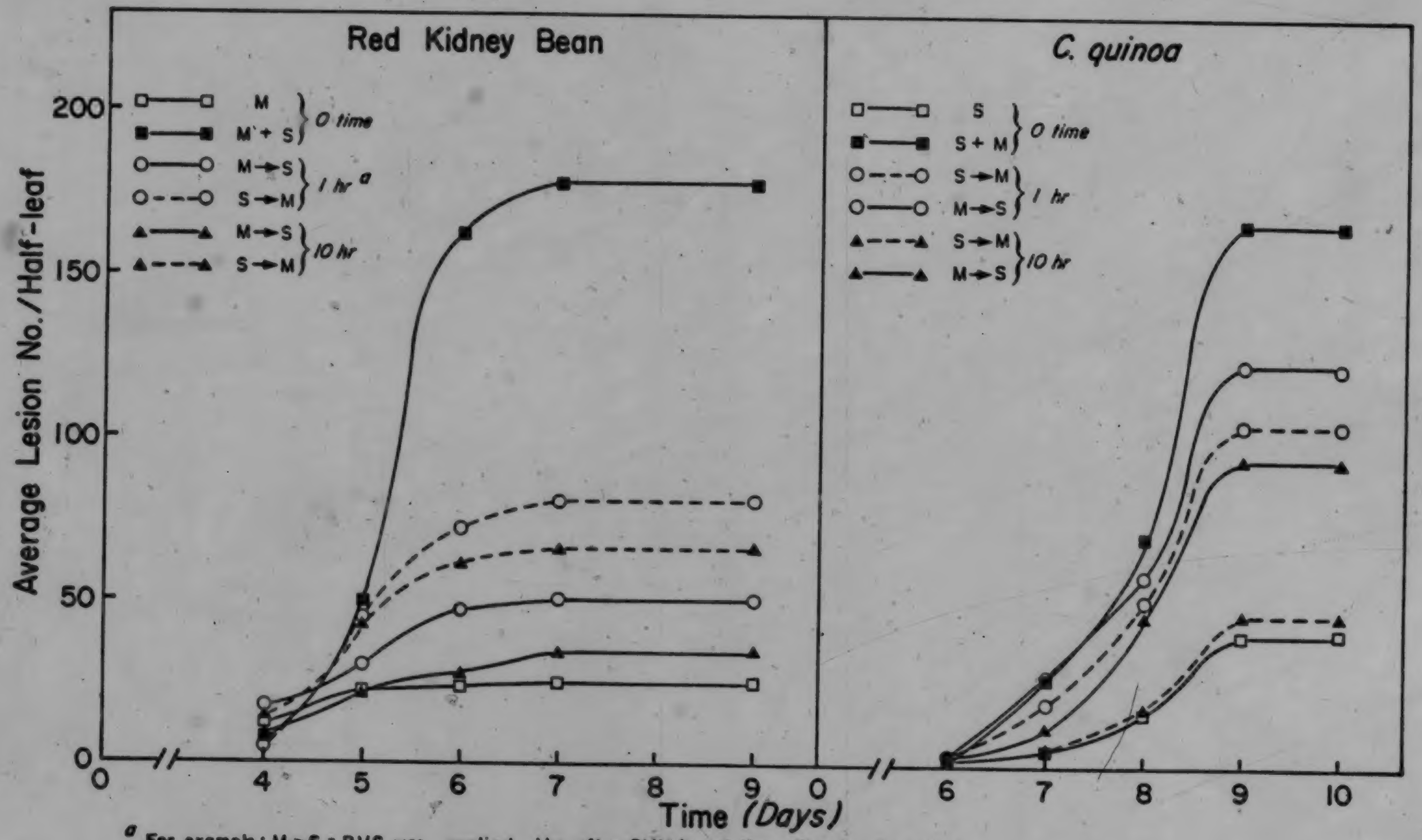
| Half-leaf no. | Lesion no./half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C[a] | P[b] | C | 1:1[c] | C | 1:5[c] | C | 1:10[c] |
| 1 | 38 | 0 | 45 | 56 | 89 | 126 | 76 | 81 |
| 2 | 51 | 0 | 38 | 92 | 115 | 225 | 84 | 94 |
| 3 | 43 | 0 | 51 | 96 | 122 | 135 | 70 | 86 |
| 4 | 33 | 0 | 53 | 67 | 103 | 129 | 72 | 72 |
| 5 | 32 | 0 | 30 | 52 | 70 | 104 | 73 | 104 |
| 6 | 37 | 0 | 48 | 46 | 81 | 157 | 81 | 258 |
| 7 | 42 | 0 | 42 | 65 | 74 | 238 | 55 | 180 |
| 8 | 29 | 0 | 34 | 39 | 49 | 103 | 47 | 156 |
| 9 | 36 | 0 | 86 | 81 | 35 | 119 | 111 | 103 |
| 10 | 50 | 0 | 100 | 158 | 44 | 129 | 126 | 145 |
| 11 | 61 | 0 | 72 | 129 | 53 | 139 | 95 | 233 |
| 12 | 28 | 0 | 60 | 117 | 40 | 98 | 92 | 129 |
| 13 | 100 | 0 | 45 | 79 | 80 | 108 | 50 | 139 |
| 14 | 103 | 0 | 72 | 100 | 103 | 141 | 65 | 101 |
| 15 | 100 | 0 | 93 | 122 | 71 | 160 | 48 | 158 |
| 16 | 94 | 0 | 55 | 83 | 48 | 148 | 40 | 69 |
| 17 | 88 | 0 | 25 | 94 | 39 | 64 | 39 | 125 |
| 18 | 111 | 0 | 28 | 103 | 40 | 86 | 45 | 137 |
| 19 | 99 | 0 | 33 | 110 | 38 | 100 | 46 | 102 |
| 20 | 64 | 0 | 30 | 112 | 32 | 87 | 33 | 137 |
| 21 | 34 | 0 | 42 | 45 | 30 | 111 | 34 | 180 |
| 22 | 50 | 0 | 44 | 41 | 41 | 129 | 43 | 131 |
| 23 | 27 | 0 | 55 | 65 | 50 | 105 | 47 | 123 |
| 24 | 29 | 0 | 45 | 54 | 54 | 76 | 43 | 78 |
| Mean | 57.5 | 0 | 51.1 | 83.6 | 62.5 | 125.7 | 63.1 | 130.0 |
| Standard error | 6.0 | 0 | 4.2 | 6.5 | 5.7 | 8.3 | 5.1 | 9.7 |
| Stimulatory index | | | 1.6 | | 2.0 | | 2.1 | |

[a] C = control, PVM only.

[b] P = 0.057 M phosphate buffer (pH 8.0) only.

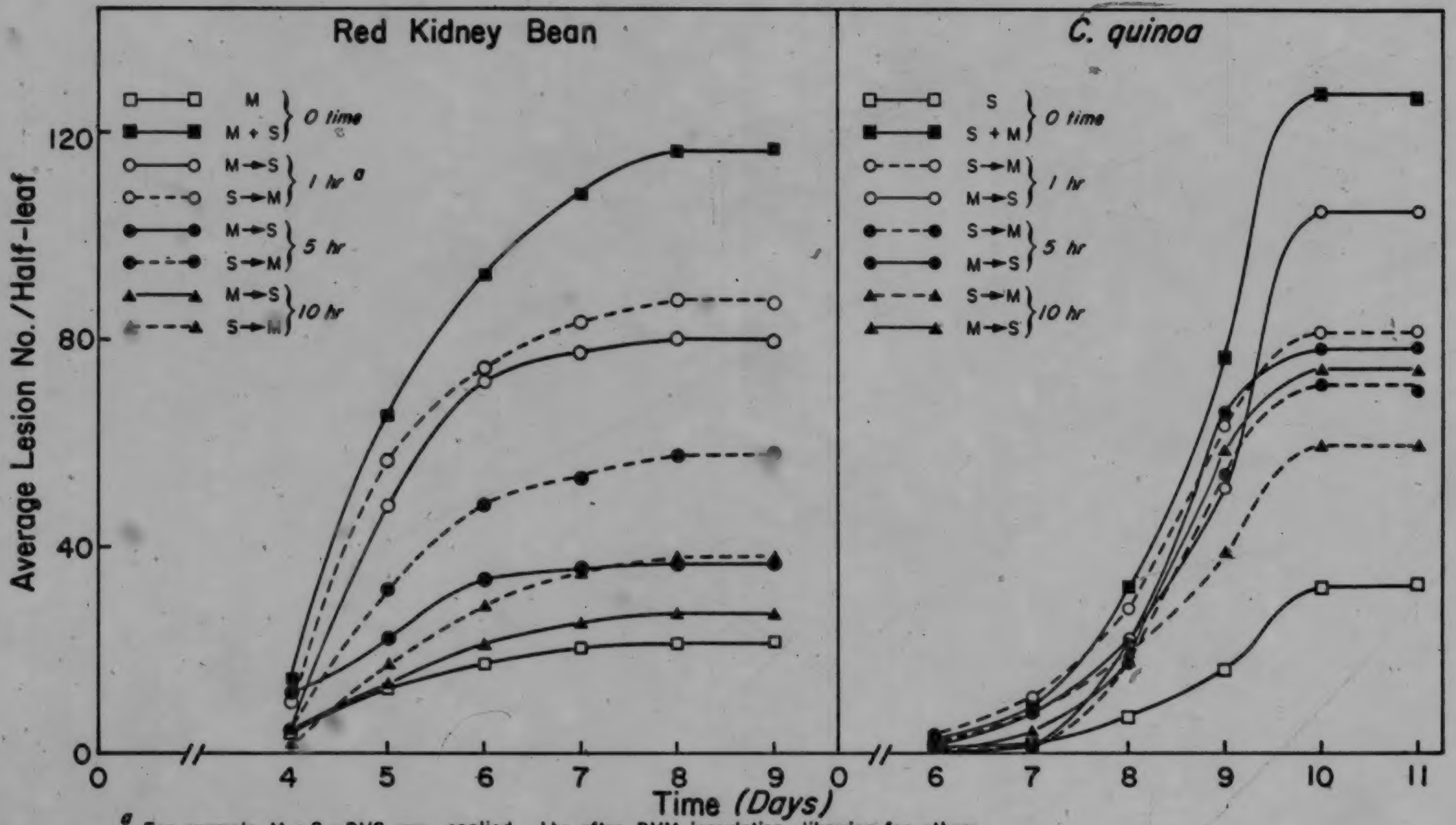
[c] 1:1 = 1 part PVM + 1 part PVS ; 1:5 = 1 part PVM + 5 parts PVS ; 1:10 = 1 part PVM + 10 parts PVS.

FIG. 13A. Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean or *Chenopodium quinoa*. *Expt. I.*



[a] For example: M → S = PVS was applied 1 hr after PVM inoculation; likewise for others.

FIG. 13B. Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean or *Chenopodium quinoa*. *Expt. 11.*



[a] For example: M→S = PVS was applied 1 hr after PVM inoculation; likewise for others.

TABLE 6. Stimulatory indexes obtained from the pre- and post-inoculation treatments with purified potato virus M(PVM) and potato virus S(PVS) in 'Red Kidney' bean or *Chenopodium quinoa*.

| | Stimulatory Index | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *EXPT. I* | | | | *EXPT. II* | | | | |
| | Control | 0 time | 1 hr | 10 hrs | Control | 0 time | 1 hr | 5 hrs | 10 hrs |
| Inocula in RKB | | | | | | | | | |
| M | 1.0 | | | | 1.0 | | | | |
| M + S | | 7.1 | | | | 5.4 | | | |
| M→S *(Pre)*[a] | | | 2.0 | 1.4 | | | 3.7 | 1.7 | 1.3 |
| S→M *(Post)*[b] | | | 3.2 | 2.7 | | | 4.0 | 2.7 | 1.7 |
| Inocula in *C. quinoa* | | | | | | | | | |
| S | 1.0 | | | | 1.0 | | | | |
| S + M | | 4.0 | | | | 4.0 | | | |
| S→M *(Pre)* | | | 2.5 | 1.1 | | | 2.5 | 2.2 | 1.8 |
| M→S *(Post)* | | | 3.0 | 2.3 | | | 3.2 | 2.4 | 2.3 |

[a] PVM inoculation was followed by PVS.

[b] PVS inoculation was followed by PVM.

In RKB, PVM lesions from all treatments started to appear 4 days after inoculation, then increased rapidly between 5 and 6 days and reached a maximum in 7 to 8 days (Figs. 13A and 13B). In *C. quinoa*, PVS lesions were visible 6 days after inoculation, then increased logarithmically during the period between 7 to 9 days and reached a maximum in 9 to 10 days (Figs. 13A and 13B).

### c. *Simultaneous separate inoculations of upper and lower epidermal layers*

There was no significant difference in the stimulatory index in simultaneous separate inoculations with PVM and PVS of the upper and lower epidermal layers of RKB and *C. quinoa* leaves (Tables 7 and 8). The results also indicated that the susceptibility of the lower epidermis of both host plants was much lower than that of the upper epidermis.

## B. Interaction Between Intact Virus, Viral RNA's, Yeast-RNA and Coat-protein

### a. *Infectivity of viral RNA's*

The UV-absorption spectra of PVM- and PVS-RNA's obtained were typical for RNA (Fig. 14). The ratio of A max./min. for PVM-RNA and PVS-RNA were 2.26 and 2.25 respectively. The infectivity of the PVS-RNA preparation was higher than that of the PVM-RNA (Fig. 15).

### b. *Interaction between intact virus and viral RNA of the counterpart*

Infectivity of the intact virus was decreased in the presence of the viral RNA of the counterpart (Tables 9 and 10). The results showed that the degree of interference was dependent on the concentration of the viral RNA of the counterpart present in the inoculum.

TABLE 7. Simultaneous seperate inoculations with purified potato virus M(PVM) and potato virus S(PVS) of the upper and lower epidermal layers of 'Red Kidney' bean.

| Expt. | Leaf no. | Lesion no./half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | M[a] | | M | | P | S | S | P |
| | | P[b] | S[c] | S | P | M | | M | |
| I | 1 | 30 | 14 | 26 | 27 | 6 | 4 | 7 | 4 |
| | 2 | 38 | 32 | 46 | 50 | 9 | 7 | 7 | 9 |
| | 3 | 18 | 29 | 13 | 10 | 2 | 5 | 6 | 6 |
| | 4 | 37 | 31 | 17 | 29 | 10 | 13 | 10 | 9 |
| | 5 | 18 | 11 | 19 | 24 | 4 | 8 | 5 | 4 |
| | 6 | 46 | 40 | 29 | 31 | 2 | 6 | 9 | 10 |
| | 7 | 15 | 18 | 16 | 25 | 3 | 4 | 3 | 3 |
| | 8 | 34 | 43 | 31 | 44 | 8 | 11 | 15 | 11 |
| | 9 | 25 | 26 | 19 | 22 | 10 | 12 | 12 | 10 |
| | 10 | 29 | 25 | 33 | 41 | 7 | 4 | 4 | 5 |
| | 11 | 27 | 22 | 16 | 13 | 5 | 2 | 9 | 4 |
| | 12 | 17 | 12 | 10 | 21 | 8 | 6 | 8 | 5 |
| | Mean | M/P(Control) = 28.0 | | | | P/M(Control) = 6.4 | | | |
| | | M/S(Test) = 24.1 | | | | S/M(Test) = 7.4 | | | |
| | Stimulatory index | Test / Control = 0.9 | | | | Test / Control = 1.2 | | | |
| II | 1 | 15 | 10 | 11 | 22 | 2 | 1 | 4 | 3 |
| | 2 | 11 | 15 | 19 | 17 | 4 | 4 | 3 | 2 |
| | 3 | 20 | 15 | 13 | 24 | 4 | 2 | 5 | 5 |
| | 4 | 35 | 40 | 30 | 30 | 3 | 3 | 6 | 4 |
| | 5 | 8 | 15 | 14 | 10 | 5 | 6 | 7 | 10 |
| | 6 | 19 | 23 | 20 | 14 | 8 | 11 | 13 | 9 |
| | 7 | 18 | 12 | 10 | 21 | 10 | 8 | 12 | 7 |
| | 8 | 24 | 21 | 27 | 17 | 7 | 6 | 10 | 8 |
| | 9 | 41 | 39 | 30 | 36 | 2 | 4 | 6 | 5 |
| | 10 | 15 | 17 | 21 | 13 | 4 | 2 | 9 | 6 |
| | 11 | 12 | 11 | 19 | 14 | 5 | 8 | 7 | 12 |
| | 12 | 28 | 14 | 16 | 19 | 14 | 9 | 10 | 7 |
| | Mean | M/P(Control) = 20.1 | | | | P/M(Control) = 6.1 | | | |
| | | M/S(Test) = 19.3 | | | | S/M(Test) = 6.5 | | | |
| | Stimulatory index | Test / Control = 1.0 | | | | Test / Control = 1.1 | | | |

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[a] 0.33 mg PVM/ml was applied to the whole upper epidermis.

[b] 0.057 M phosphate buffer (pH 8) was applied to one-half of the lower epidermis.

[c] 0.40 mg PVS/ml was applied to one-half of the lower epidermis. Similar scheme applies for other inoculations.

TABLE 8. Simultaneous seperate inoculations with purified potato virus S(PVS) and potato virus M (PVM) of the upper and lower epidermal layers of *Chenopodium quinoa.*

| Expt. | Leaf no. | Lesion no./half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | S[a] | | S | | P | M | M | P |
| | | P[b] | M[c] | M | P | S | | S | |
| I | 1 | 31 | 33 | 50 | 44 | 23 | 28 | 26 | 14 |
| | 2 | 41 | 48 | 45 | 38 | 31 | 44 | 53 | 35 |
| | 3 | 49 | 67 | 60 | 46 | 15 | 18 | 22 | 21 |
| | 4 | 77 | 63 | 59 | 60 | 27 | 40 | 25 | 34 |
| | 5 | 70 | 76 | 79 | 63 | 42 | 51 | 35 | 50 |
| | 6 | 50 | 59 | 42 | 40 | 22 | 16 | 28 | 18 |
| | 7 | 33 | 47 | 32 | 30 | 26 | 23 | 24 | 24 |
| | 8 | 60 | 81 | 42 | 49 | 25 | 29 | 23 | 31 |
| | 9 | 47 | 30 | 31 | 30 | 32 | 27 | 34 | 20 |
| | 10 | 30 | 21 | 25 | 44 | 21 | 25 | 16 | 19 |
| | 11 | 23 | 32 | 20 | 25 | 22 | 17 | 15 | 20 |
| | 12 | 37 | 30 | 34 | 37 | 24 | 31 | 27 | 25 |
| | Mean | S/P(Control) = 43.9<br>S/M(Test) = 46.1 | | | | P/S(Control) = 25.9<br>M/S(Test) = 28.2 | | | |
| | Stimulatory index | Test/Control = 1.1 | | | | Test/Control = 1.1 | | | |
| II | 1 | 21 | 35 | 40 | 30 | 27 | 25 | 31 | 20 |
| | 2 | 51 | 37 | 45 | 38 | 30 | 20 | 22 | 17 |
| | 3 | 44 | 39 | 54 | 47 | 25 | 26 | 31 | 29 |
| | 4 | 30 | 29 | 31 | 42 | 45 | 52 | 47 | 34 |
| | 5 | 29 | 27 | 36 | 44 | 37 | 40 | 37 | 41 |
| | 6 | 64 | 60 | 70 | 89 | 49 | 60 | 59 | 53 |
| | 7 | 70 | 54 | 66 | 51 | 19 | 30 | 25 | 26 |
| | 8 | 49 | 39 | 63 | 62 | 32 | 18 | 39 | 26 |
| | 9 | 80 | 95 | 80 | 93 | 35 | 38 | 45 | 28 |
| | 10 | 21 | 26 | 26 | 33 | 20 | 26 | 38 | 26 |
| | 11 | 53 | 47 | 50 | 72 | 45 | 70 | 56 | 61 |
| | 12 | 35 | 33 | 46 | 70 | 22 | 33 | 20 | 37 |
| | Mean | S/P(Control) = 50.8<br>S/M(Test) = 47.0 | | | | P/S(Control) = 32.7<br>M/S(Test) = 37.0 | | | |
| | Stimulatory index | Test/Control = 0.9 | | | | Test/Control = 1.1 | | | |

[a] 0.16 mg of PVS/ml was applied to the whole upper epidermis.

[b] 0.057 M phosphate buffer (pH 8) was applied to one-half of the lower epidermis.

[c] 1.67 mg PVM/ml was applied to one-half of the lower epidermis. Similar scheme applies for other inoculations.

FIG. 14. The UV spectra of ribonucleic acids (RNA's) respectively isolated from potato virus M(PVM) and potato virus S (PVS).

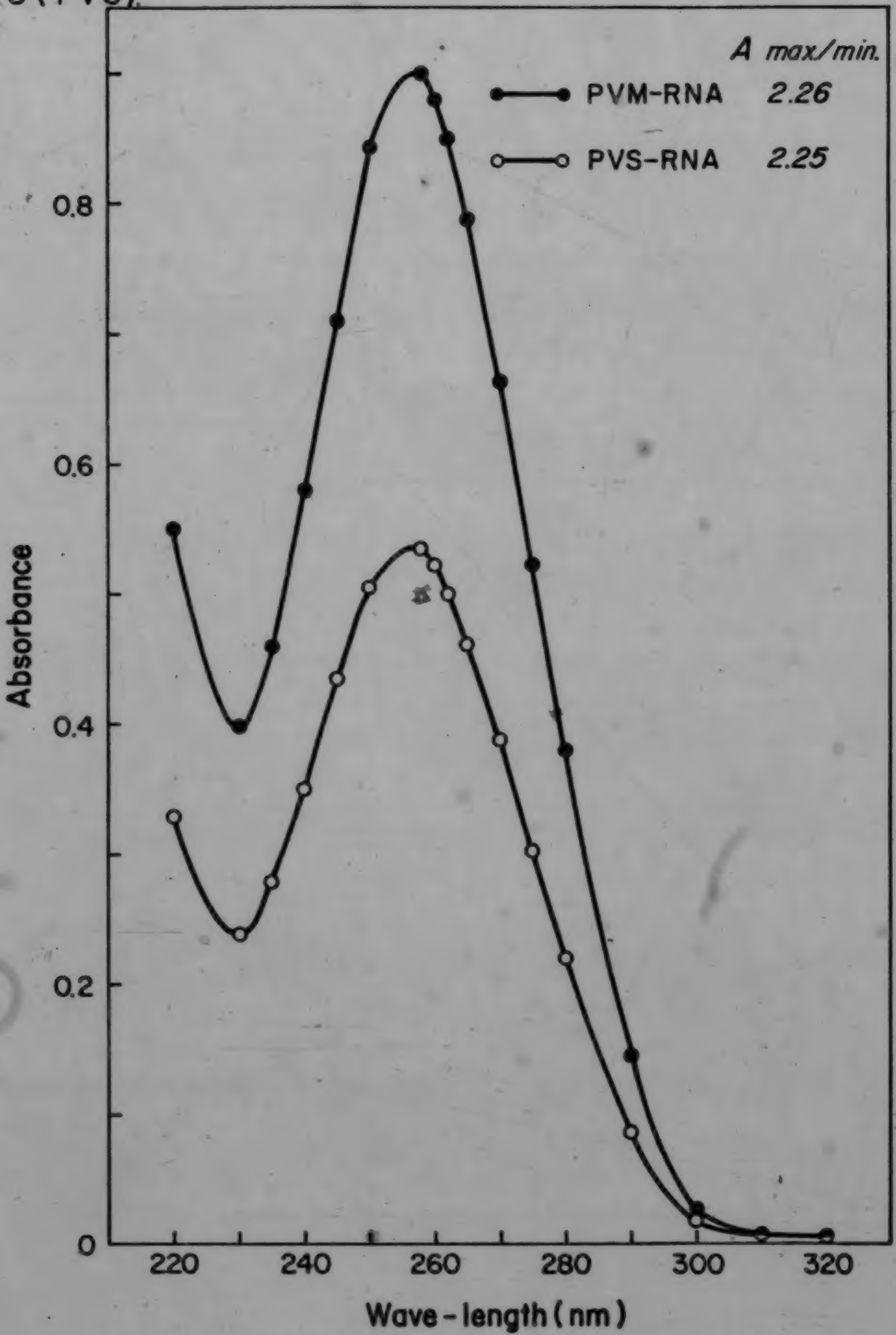


FIG. 15. The dilution curves of the infectivity of ribonucleic acids (RNA's) respectively isolated from potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean and *Chenopodium quinoa*.

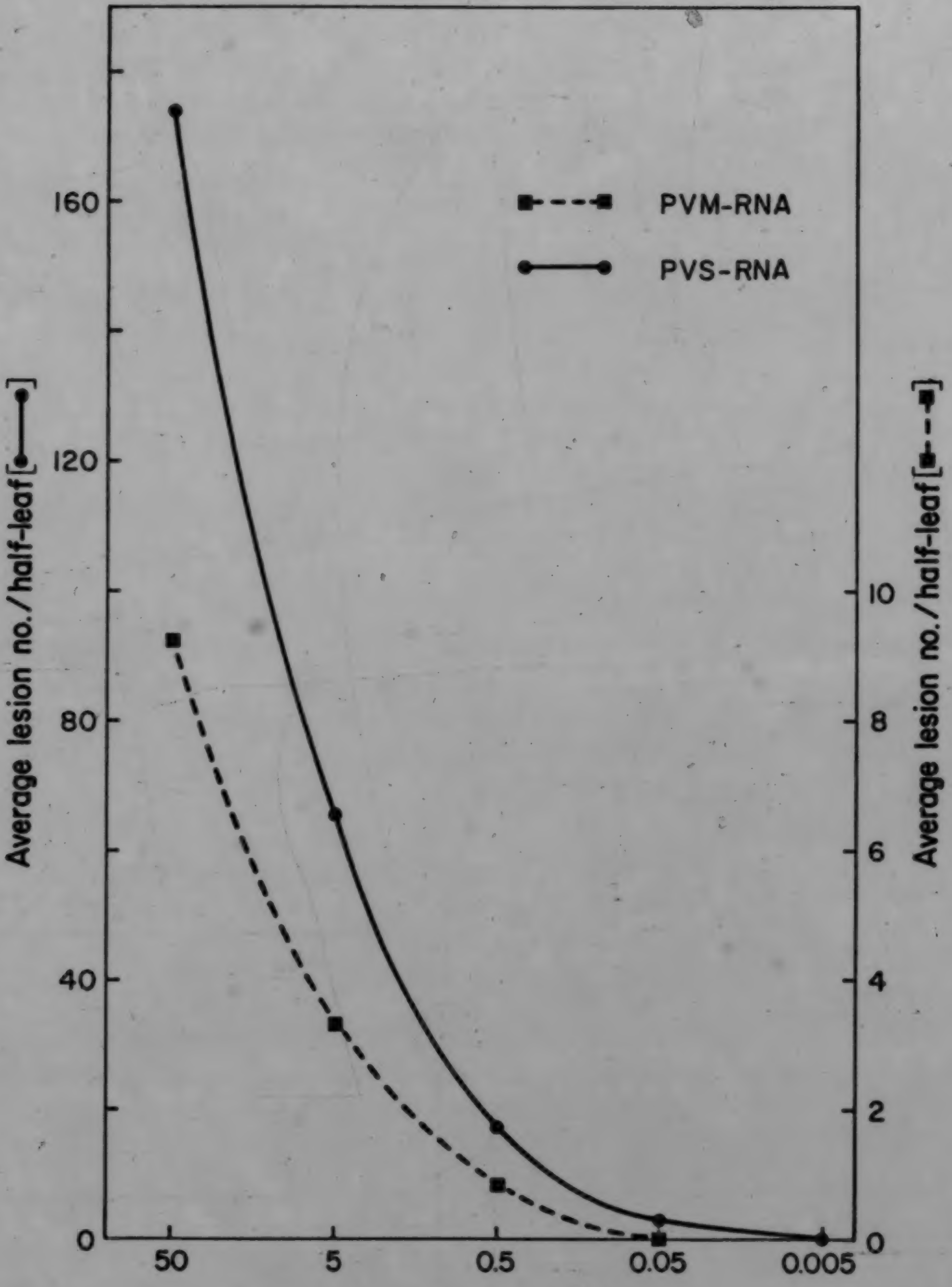


TABLE 9. Interaction between intact potato virus M (PVM) and potato virus S (PVS)-ribonucleic acid (RNA) in 'Red Kidney' bean.

| Half-leaf no. | Lesion no./half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Expt. I* | | | | *Expt. II* | | | |
| | C[a] | $T_1$[b] | C[a] | $T_2$[b] | C[a] | $T_1$[c] | C[a] | $T_2$[c] |
| 1 | 50 | 43 | 75 | 30 | 35 | 14 | 70 | 38 |
| 2 | 81 | 28 | 68 | 35 | 105 | 35 | 92 | 21 |
| 3 | 62 | 22 | 116 | 67 | 64 | 20 | 51 | 24 |
| 4 | 61 | 15 | 135 | 82 | 56 | 23 | 80 | 30 |
| 5 | 79 | 32 | 143 | 62 | 73 | 17 | 68 | 32 |
| 6 | 100 | 23 | 84 | 43 | 27 | 30 | 60 | 46 |
| 7 | 79 | 18 | 58 | 71 | 43 | 18 | 62 | 35 |
| 8 | 75 | 21 | 68 | 52 | 59 | 25 | 42 | 29 |
| 9 | 81 | 40 | 100 | 46 | 37 | 10 | 50 | 40 |
| 10 | 71 | 33 | 106 | 44 | 75 | 19 | 45 | 26 |
| 11 | 61 | 27 | 74 | 27 | 108 | 33 | 36 | 30 |
| 12 | 111 | 24 | 54 | 26 | 54 | 16 | 120 | 59 |
| Mean | 75.9 | 27.2 | 90.1 | 48.8 | 61.3 | 21.7 | 64.7 | 34.2 |
| Standard error | 4.9 | 2.5 | 8.6 | 5.3 | 7.4 | 2.2 | 6.9 | 3.0 |
| Stimulatory index | 0.4 | | 0.5 | | 0.4 | | 0.5 | |

[a] Control: 1.1 mg/ml PVM.

[b] Test *(I)*: $T_1$ = 1.1 mg/ml PVM + 5 *A* units of PVS-RNA at *258 nm*;
$T_2$ = 1.1 mg/ml PVM + 0.5 *A* units of PVS-RNA at *258 nm*.

[c] Test *(II)*: $T_1$ = 1.1 mg/ml PVM + 6 *A* units of PVS-RNA at *258 nm*;
$T_2$ = 1.1 mg/ml PVM + 0.6 *A* units of PVS-RNA at *258 nm*.

TABLE 10. Interaction between intact potato virus S (PVS) and potato virus M (PVM)-ribonucleic acid (RNA) in *Chenopodium quinoa*.

| Half-leaf no. | Lesion no / half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Expt. I* | | | | *Expt. II* | | | |
| | C[a] | $T_1$[b] | C[a] | $T_2$[b] | C[c] | $T_1$[d] | C[c] | $T_2$[d] |
| 1 | 52 | 11 | 57 | 36 | 36 | 11 | 24 | 10 |
| 2 | 61 | 45 | 60 | 42 | 77 | 20 | 89 | 58 |
| 3 | 40 | 20 | 39 | 29 | 45 | 11 | 22 | 25 |
| 4 | 77 | 31 | 71 | 52 | 29 | 19 | 48 | 12 |
| 5 | 26 | 26 | 52 | 27 | 50 | 7 | 19 | 33 |
| 6 | 45 | 30 | 33 | 24 | 39 | 25 | 66 | 13 |
| 7 | 59 | 40 | 28 | 22 | 50 | 12 | 67 | 14 |
| 8 | 39 | 14 | 31 | 38 | 48 | 35 | 40 | 22 |
| 9 | 43 | 23 | 50 | 40 | 37 | 10 | 33 | 43 |
| 10 | 51 | 33 | 101 | 71 | 28 | 8 | 75 | 17 |
| 11 | 48 | 24 | 67 | 20 | 43 | 9 | 66 | 30 |
| 12 | 90 | 13 | 54 | 43 | 25 | 15 | 29 | 29 |
| Mean | 52.6 | 25.8 | 53.6 | 37.0 | 42.3 | 15.2 | 47.3 | 25.5 |
| Standard error | 5.0 | 3.1 | 5.9 | 4.2 | 4.0 | 2.4 | 6.7 | 4.1 |
| Stimulatory index | 0.5 | | 0.7 | | 0.4 | | 0.5 | |

[a] Control *(I)*: 0.24 mg/ml PVS.

[b] Test *(I)*: $T_1$ = 0.24 mg/ml PVS + 30 $A$ units of PVM-RNA at *258nm*;
$T_2$ = 0.24 mg/ml PVS + 3.0 $A$ units of PVM-RNA at *258nm*.

[c] Control *(II)*: 0.16 mg/ml PVS.

[d] Test *(II)*: $T_1$ = 0.16 mg/ml PVS + 30 $A$ units of PVM-RNA at *258 nm*;
$T_2$ = 0.16 mg/ml PVS + 3.0 $A$ units of PVM-RNA at *258nm*.

The results also indicated the reductions in the lesion number due to the presence of the RNA of the counterpart were approximately 50 to 60% for PVM in RKB (Table 9) and 30 to 60% for PVS in *C. quinoa* (Table 10).

### c. *Simultaneous inoculation with PVM- and PVS-RNA's*

Only slight interference was induced by the viral RNA of the counterpart. The results showed that about a 10% inhibition of the PVM-RNA infectivity occurred in RKB (Table 11) and about 20 to 30% inhibition of PVS-RNA infectivity in *C. quinoa* (Table 12).

### d. *Simultaneous inoculation with intact virus and viral coat-protein of the counterpart*

In both cases, the infectivity of the test viruses was slightly higher in the presence of the coat-protein of the counterpart in the inoculum. On the whole, as the stimulatory index indicates, about 10 to 30% increases in local lesion number were obtained (Tables 13 and 14). The inoculum containing coat-protein only did not produce any lesion.

### e. *Detection of endogeneous RNase activity*

In both cases the infectivity of PVM- and PVS-RNA's was higher as the concentration of the intact viral counterpart was reduced (Fig. 16 and 17). The activity of the endogenous RNase apparently diminished upon diluting the preparation containing the intact viral counterpart between the dilutions $10^{-2}$ to $10^{-4}$.

### f. *Effect of endogenous RNase on PVS-RNA infectivity in the presence of yeast-RNA*

The infectivity of PVS-RNA was retained in the presence of yeast-RNA in the inoculum (Table 15). As the concentration of yeast-RNA in the inocula increased, there were apparent decreases in

TABLE 11. Simultaneous application of potato virus M (PVM)- and potato virus S (PVS)-ribonucleic acids (RNA's) to 'Red Kidney' bean.

| Half-leaf no. | Lesion no./half-leaf | | | |
|---|---|---|---|---|
| | *Expt. I* | | *Expt. II* | |
| | C[a] | T[b] | C[a] | T[c] |
| 1 | 3 | 4 | 5 | 6 |
| 2 | 8 | 6 | 6 | 3 |
| 3 | 18 | 15 | 15 | 14 |
| 4 | 11 | 10 | 12 | 10 |
| 5 | 5 | 6 | 6 | 7 |
| 6 | 4 | 3 | 3 | 3 |
| 7 | 8 | 5 | 10 | 10 |
| 8 | 6 | 5 | 4 | 3 |
| 9 | 10 | 10 | 8 | 7 |
| 10 | 15 | 12 | 11 | 9 |
| 11 | 9 | 8 | 12 | 11 |
| 12 | 11 | 13 | 9 | 9 |
| Mean | 9.0 | 8.1 | 8.4 | 7.7 |
| Standard error | 1.3 | 1.1 | 1.1 | 1.0 |
| Stimulatory index | 0.9 | | 0.9 | |

[a] Control: 40 *A* units of PVM-RNA at *258 nm.*

[b] Test*(I)*:40 *A* units of PVM-RNA+6 *A* units of PVS-RNA, all at *258 nm.*

[c] Test*(II)*:40 *A* units of PVM-RNA+12 *A* units of PVS-RNA, all at *258 nm.*

TABLE 12. Simultaneous application of potato virus S (PVS) - and potato virus M (PVM) - ribonucleic acids (RNA's) to *Chenopodium quinoa*.

| Half-leaf no. | Lesion no. / half-leaf | | | |
|---|---|---|---|---|
| | Expt. I | | Expt. II | |
| | C[a] | T[b] | C[a] | T[c] |
| 1 | 109 | 115 | 129 | 78 |
| 2 | 59 | 42 | 69 | 45 |
| 3 | 222 | 200 | 212 | 208 |
| 4 | 178 | 160 | 208 | 169 |
| 5 | 119 | 78 | 152 | 92 |
| 6 | 115 | 88 | 134 | 77 |
| 7 | 152 | 112 | 175 | 121 |
| 8 | 144 | 69 | 114 | 64 |
| 9 | 89 | 51 | 92 | 51 |
| 10 | 120 | 91 | 149 | 106 |
| 11 | 169 | 115 | 211 | 122 |
| 12 | 155 | 99 | 203 | 107 |
| Mean | 135.9 | 101.7 | 154.0 | 103.3 |
| Standard error | 12.6 | 12.8 | 14.1 | 13.8 |
| Stimulatory index | 0.8 | | 0.7 | |

[a] Control: 6 A units of PVS-RNA at *258 nm.*

[b] Test*(I)*: 6 A units of PVS-RNA + 40 A units of PVM-RNA, all at *258 nm.*

[c] Test*(II)*: 6 A units of PVS-RNA + 30 A units of PVM-RNA, all at *258 nm.*

TABLE 13. Simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) coat-protein to 'Red Kidney' bean.

| Half-leaf no. | Lesion no. / half-leaf | | | | | |
|---|---|---|---|---|---|---|
| | *Expt. I* | | | *Expt. II* | | |
| | C[a] | T[b] | Coat-protein only[c] | C[a] | T[d] | Coat-protein only[c] |
| 1 | 101 | 118 | 0 | 31 | 30 | 0 |
| 2 | 39 | 96 | 0 | 72 | 89 | 0 |
| 3 | 27 | 39 | 0 | 63 | 56 | 0 |
| 4 | 78 | 51 | 0 | 30 | 47 | 0 |
| 5 | 41 | 39 | 0 | 23 | 45 | 0 |
| 6 | 72 | 68 | 0 | 69 | 41 | 0 |
| 7 | 80 | 106 | 0 | 45 | 40 | 0 |
| 8 | 66 | 73 | 0 | 74 | 95 | 0 |
| 9 | 33 | 40 | 0 | 32 | 79 | 0 |
| 10 | 34 | 66 | 0 | 73 | 78 | 0 |
| 11 | 41 | 42 | 0 | 40 | 49 | 0 |
| 12 | 37 | 72 | 0 | 34 | 30 | 0 |
| Mean | 54.1 | 67.5 | 0 | 48.8 | 56.6 | 0 |
| Standard error | 6.9 | 7.9 | 0 | 5.7 | 6.6 | 0 |
| Stimulatory index | 1.3 | | | 1.2 | | |

[a] Control: 1.1 mg/ml PVM.

[b] Test *(I)*: 1.1 mg/ml PVM + 0.5 *A* units of PVS coat-protein at *280 nm.*

[c] Same concentration was used as in the *Test* inoculum.

[d] Test *(II)*: 1.1 mg/ml PVM+ 1.0 *A* units of PVS coat-protein at *280 nm.*

TABLE 14. Simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) coat-protein to *Chenopodium quinoa*.

| Half-leaf no. | Lesion no. / half leaf | | | | | |
|---|---|---|---|---|---|---|
| | *Expt. I* | | | *Expt. II* | | |
| | C[a] | T[b] | Coat-protein only[c] | C[a] | T[d] | Coat-protein only[c] |
| 1 | 56 | 70 | 0 | 54 | 88 | 0 |
| 2 | 126 | 171 | 0 | 43 | 49 | 0 |
| 3 | 49 | 73 | 0 | 101 | 64 | 0 |
| 4 | 84 | 86 | 0 | 80 | 123 | 0 |
| 5 | 46 | 66 | 0 | 168 | 121 | 0 |
| 6 | 100 | 97 | 0 | 94 | 158 | 0 |
| 7 | 59 | 49 | 0 | 69 | 61 | 0 |
| 8 | 93 | 126 | 0 | 72 | 78 | 0 |
| 9 | 69 | 113 | 0 | 93 | 79 | 0 |
| 10 | 76 | 132 | 0 | 91 | 114 | 0 |
| 11 | 141 | 93 | 0 | 116 | 135 | 0 |
| 12 | 111 | 116 | 0 | 120 | 93 | 0 |
| Mean | 84.2 | 99.3 | 0 | 91.8 | 96.9 | 0 |
| Standard error | 8.9 | 9.9 | 0 | 9.6 | 9.6 | 0 |
| Stimulatory index | 1.2 | | | 1.1 | | |

[a] Control: 0.3 mg/ml PVS.

[b] Test *(I)*: 0.3 mg/ml PVS + 1.0 *A* units of PVM coat-protein at *280 nm*.

[c] Same concentration was used as in the *Test* inoculum.

[d] Test *(II)*: 0.3 mg/ml PVS + 1.5 *A* units of PVM coat-protein at *280 nm*.

FIG. 16. Effect of endogenous ribonuclease associated with intact potato virus S (PVS) on the infectivity of potato virus M (PVM)-ribonucleic acid (RNA) in 'Red Kidney' bean.

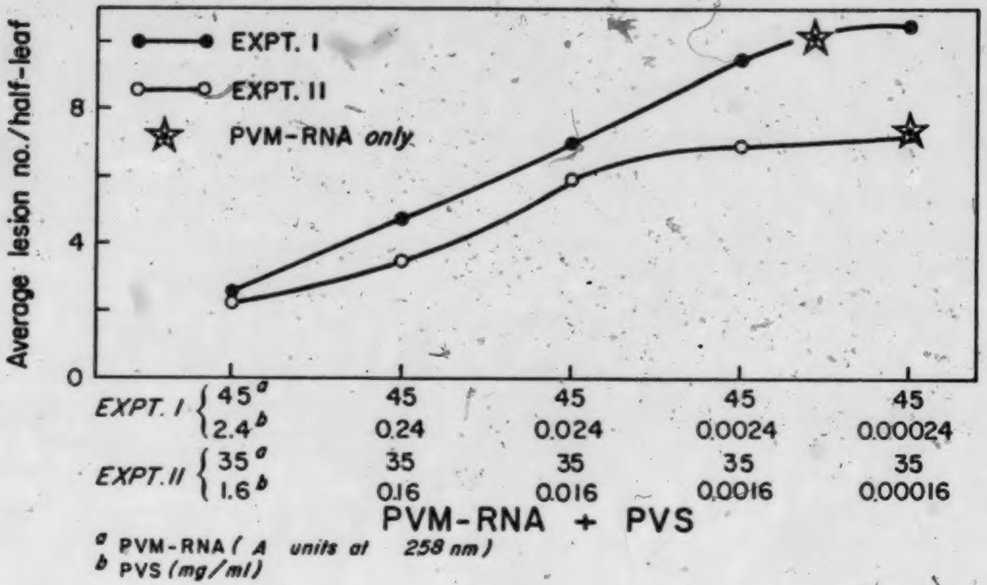


FIG. 17. Effect of endogenous ribonuclease associated with intact potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in *Chenopodium quinoa.*

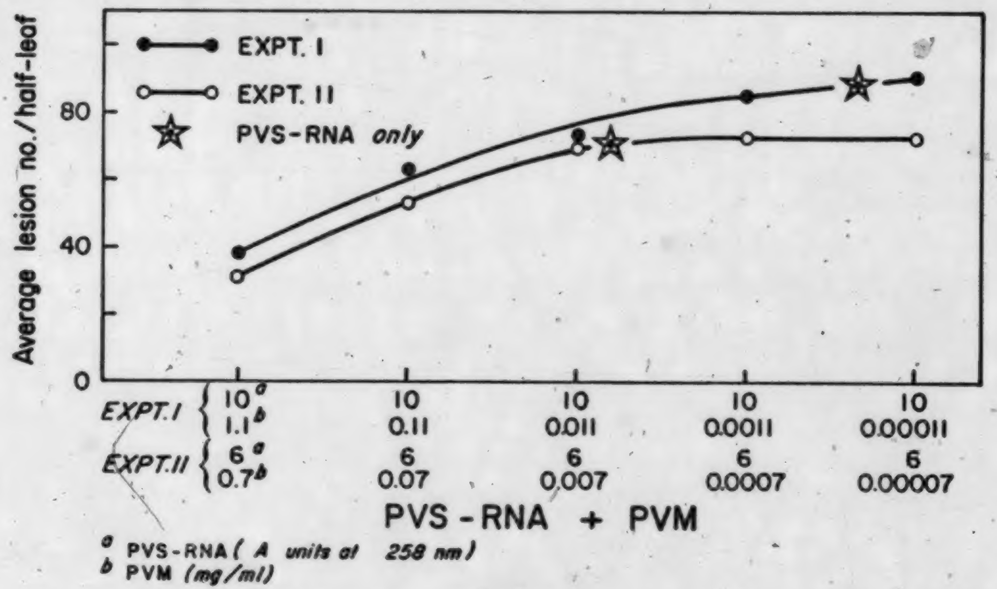


TABLE 15. The effect of endogenous ribonuclease associated with the purified potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in *Chenopodium quinoa* in the presence of yeast-RNA.

| Half-leaf no. | Lesion no./half-leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Expt. I* | | | | *Expt. II*[c] | | | |
| | C[a] | $T_1$[b] | $T_2$ | $T_3$ | C | $T_1$ | $T_2$ | $T_3$ |
| 1 | 27 | 6 | 8 | 21 | 115 | 56 | 60 | 69 |
| 2 | 30 | 7 | 10 | 16 | 134 | 42 | 79 | 106 |
| 3 | 67 | 17 | 16 | 35 | 63 | 17 | 23 | 38 |
| 4 | 56 | 11 | 21 | 51 | 49 | 14 | 32 | 30 |
| 5 | 71 | 16 | 33 | 58 | 102 | 44 | 50 | 61 |
| 6 | 99 | 20 | 24 | 42 | 91 | 31 | 73 | 65 |
| 7 | 24 | 5 | 9 | 16 | 146 | 50 | 102 | 135 |
| 8 | 38 | 8 | 18 | 20 | 177 | 63 | 81 | 89 |
| 9 | 27 | 6 | 19 | 14 | 68 | 36 | 24 | 34 |
| 10 | 49 | 6 | 21 | 29 | 58 | 20 | 40 | 37 |
| 11 | 63 | 11 | 26 | 24 | 43 | 26 | 49 | 59 |
| 12 | 52 | 12 | 30 | 29 | 75 | 36 | 62 | 92 |
| Mean | 50.3 | 10.4 | 19.6 | 29.6 | 93.4 | 36.3 | 56.3 | 67.9 |
| Standard error | 6.5 | 1.4 | 2.3 | 4.1 | 12.2 | 4.5 | 7.1 | 9.4 |
| Infectivity index | 1.0 | 0.2 | 0.4 | 0.6 | 1.0 | 0.4 | 0.6 | 0.7 |

[a] Control: 5 *A* units of PVS-RNA at *258 nm.*

[b] Test *(I)*: $T_1$ = 5 *A* units of PVS-RNA + 1.1 mg/ml PVM + phosphate buffer (0.057 M, pH 8).

$T_2$ = 5 *A* units of PVS-RNA + 1.1 mg/ml PVM + 25 *A* units of yeast-RNA at *258 nm.*

$T_3$ = 5 *A* units of PVS-RNA + 1.1 mg/ml PVM + 50 *A* units of yeast-RNA at *258 nm.*

[c] 10 *A* units of PVS-RNA were used, the rest being the same.

inhibition of the infectivity of PVS-RNA. However, the infectivity of mixed inocula never exceeded that of PVS-RNA alone.

## DISCUSSION

In the mixed inoculation experiments, both PVM and PVS produced higher lesion numbers in their respective local lesion hosts in the presence of their counterparts. A plausible explanation for the increase in the lesion number might be that the counterparts, possibly their coat-proteins, played a part in activating a mechanism essential to initiate an infection, although they were unable to initiate infection by themselves in the same plants. The increase in infectivity, alternatively, may be due to a physical phenomenon. Although bioassay was the most sensitive method for testing virus, the production of a single local lesion requires about 50,000 to 1 million particles in the inoculum (Steere, 1955, 1956; Wildman, 1959). These data implied that the presence of a certain number of virus particles was essential at an infectible site to transform it to an infective centre. A hypothesis which may be worth considering is that the counterpart in the virus mixture acts in such a way that the efficiency of infection by the test virus is increased at the infectible site, thus enabling the fewer test virus particles to initiate transformation of infectible sites. This kind of stimulation would not produce a maximum effect when a test virus fully activated by the presence of the counterpart in the inoculum reaches a threshold beyond which the former becomes excessive for the infection of a host leaf where the inoculable area is limited. This explanation seems applicable to PVS in *C. quinoa* since the increases of PVM beyond the certain level did not proportionally increase the lesion number (Fig. 12). However, in the experiment involving a host which has comparatively larger leaf area and a virus preparation of low specific infectivity, the presence of the counterpart induced a pronounced stimulatory effect which was somewhat proportional to the amount of the counterpart added to the intact virus samples (Fig. 11).

Obtaining a similar stimulatory effect, Fulton (1962) reported that *Dolichos biflorus*, which was susceptible only to the H strain and not to the G strain of sour cherry necrotic ringspot virus, produced significantly more lesions than the control when inoculated simultaneously with a mixture of the H and G strains. Even after heat- or UV-inactivation the G strain provided the same stimulatory effect. Thus, the author suggested that the stimulation of infection required more than one particle.

In the pre- and post-inoculation experiments, the counterpart stimulated the infection of the test virus to a certain extent (Figs. 13A and 13B; Table 6). Therefore, it is safe to assume from these data that some pre-inoculated virus particles remained stable until such time that it could interact with the post-inoculated virus particles. When a total number of virus particles supplemented by either a test virus or its counterpart reaches a level that is sufficient to support infection, even a few infectious test virus particles that were present in the inoculum could achieve successful infection. This stimulatory effect, however, diminished after an interval of 10 hr between applications of the test virus and its counterpart.

The results from the experiments concerning 'simultaneous' separate inoculations of upper and lower epidermal cells indicated that the separate applications of the two viruses did not induce the stimulatory effect. Thus it was clearly shown that the stimulatory effect resulted mainly from simultaneous application of the two viruses (Figs. 11 and 12) and from sequential application involving pre- and post-inoculations to some extent (Fig. 13; Table 6).

Unlike the interaction between intact viruses, simultaneous inoculation of intact virus and the viral RNA of its counterpart resulted in decreases in the local lesion number incited by the test viruses (Tables 9 and 10). Similarly, experiments with a mixture of viral RNA's yielded an inhibitory effect (Tables 11 and 12). This result suggested that genetic recombination of these two viruses was unlikely and that there was no genetic interaction between PVM and PVS even at RNA level. Therefore, the stimulation of infection using the mixed-virus-system was achieved possibly through the physical interaction of the particles between the intact viruses.

The fact that the lesion numbers obtained from the mixed-inocula of the RNA and intact viral counterpart were essentially inversely proportional to the concentration of the counterpart can be explained by the presence of RNase in the final purified virus preparations (Figs. 16 and 17). This activity of the endogenous RNase could be counteracted by adding yeast-RNA to the inoculum (Table 15). Whitfeld and Williams (1963) earlier detected a similar RNase activity present in their purified TMV preparations, and its optimal activity ranged from pH 8.5 to 9.0. In this study, a relatively high RNase activity was expected since pH 8.0 was maintained in all inocula used.

Kaper and Siberg (1969) also found the persistent presence of a trace of RNase in the purified TYMV preparations.

# CHAPTER VI

# CONCLUDING REMARKS

The primary objectives of investigating the methods of purification and bioassay of PVM and PVS were to obtain highly purified virus preparations and to obtain information regarding factors influencing quantitative evaluation of their infectivity so that the interaction between the two viruses can be studied accurately.

Although a certain amount of information concerning the physical properties of both viruses was obtained, the characterization of the purified viruses was by no means complete. Since relatively high virus yield can be obtained by the method used in this study, efforts can be continued along this line in the future. Studies of virus components such as protein and nucleic acid can be particularly rewarding, since both PVM and PVS have been only poorly characterized in this regard. The PVM preparations obtained in this investigation had rather low specific infectivity and the sedimentation coefficient values lower than expected. This result indicates that the PVM isolate (AP-1) used in this study is perhaps more susceptible to inactivation than the PVS isolate (A) during purification. The relatively low infectivity of the RNA isolated from PVM (Chapter V) may be due to the same reason. This situation could be remedied probably by incorporating certain chelating agents in the purification procedure, in particular at the time of sap extraction (Hampton and Fulton, 1959, 1961; Hiruki, 1964).

The mechanism of virus precipitation by the PEG-NaCl method is unknown even though several hypotheses have been proposed. The precipitation of viruses by PEG was dependent on the difference in the charge on the surface of the virus particles (Polson et al., 1964; Albrechtová and Klír, 1970). Therefore, the PEG solubility could be controlled by adjusting the concentration or pH of the buffer solution used as a suspending medium (Clark and Lister, 1971; Reddy and Black, 1973). The PEG precipitation of proteins and viruses was comparable to the dehydrating effect of alcohol or acetone by removing the water molecules from the hydration envelope of the protein, causing a disturbance of the dielectric constant of the medium (Chun et al., 1967; Clark and Lister, 1971). As a result, the steric relationships of the hydrophobic and hydrophilic groups of the proteins were altered which led to the precipitation of the protein. Leberman (1966) proposed that virus precipitation by PEG could be due to a simple coacervation whereby a spontaneous separation of the highly hydrated polymers resulted in the formation of two phases. Iverius and Laurent (1967) hypothesized that PEG sterically excluded the virus particles from the solvent by creating a solubility limit for the virus, while Juckes (1971) suggested the PEG precipitation resembled the salting-out phenomenon.

In the present study the interaction between PVM and PVS is surprisingly different from any of the known phenomena. Thus a number of hypotheses that have been proposed (Bawden and Kassanis, 1945; Kavanau, 1949; Fulton, 1951a,b; Best 1954a,b; Damirdagh and Ross, 1967; Matthews, 1970; Wenzel, 1971; Kassanis et al., 1974) are not useful in explaining the results. Although the counterpart viruses, alone or in association with the test viruses, are unable to establish any detectable infection in the local lesion hosts of the test viruses, they seem to participate in infection by the test viruses in an unknown manner and contribute to the increased efficiency of infection by the latter. This stimulation is highest in its effect, when the two viruses are mixed and applied simultaneously to the test plants. However, the same effect did not occur when each of them was applied separately from the upper and lower sides of the leaf tissue, or when the RNA's from these two viruses were mixed and applied to the test plants or when the intact test virus was mixed with the RNA of the counterpart and applied. The fact that the stimulatory effect of the isolated coat-proteins is considerably low when compared with that of the intact viruses may suggest the importance of protein configuration or certain requirement(s) for their molecular size. Further careful investigations, including isotopic labelling of virus and its components coupled with the immunofluorescence technique which can be effectively applicable to protoplast systems, will undoubtedly shed light to the mechanism(s) of this extremely interesting type of virus interaction.

# PHOTOGRAPHIC PLATES

PLATE 1. Systemic and local lesion hosts of potato viruses M and S.

A. 50-day old systemic host, *Solanum tuberosum* L. potato plants.

M: A 'King Edward' potato plant infected with potato virus M (PVM) (Alberta isolate AP-1) showing symptoms of mild stunting.

H: A healthy 'Netted Gem' (Russet Burbank) plant in bloom.

S: A 'Netted Gem' potato plant infected with potato virus S (PVS) (Canadian isolate 'A') showing mild stunting at the blossom stage.

B. The primary leaf of *Phaseolus vulgaris* L. cv. 'Red Kidney' showing brown necrotic local lesions 8 days after inoculation with PVM. The inset shows an enlarged portion of the infected leaf.

C. A *Chenopodium quinoa* Willd. leaf 9 days after inoculation with PVS, showing local lesions with a necrotic centre and a chlorotic periphery.

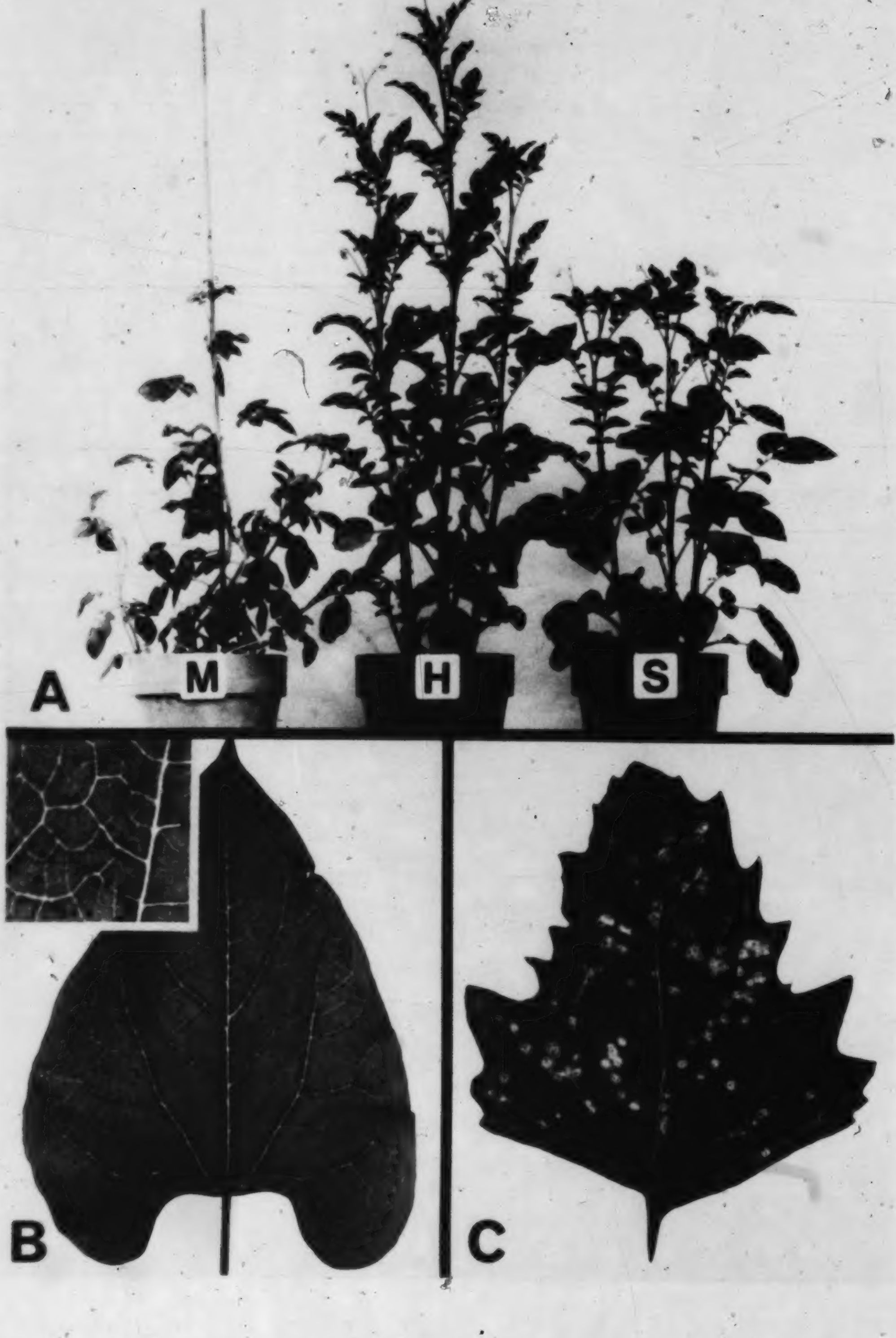

A
M
H
S
B
C

PLATE 2. Electron micrographs and length-distribution of potato virus M (PVM) obtained by the polyethylene glycol-sodium chloride purification method.

A & B. PVM particles stained with 2% sodium phosphotungstate, pH 7.0.

C. A histogram showing the length-distribution based on a measurement of 300 PVM particles.

D. Negatively stained PVM particles observed in a leaf-dip preparation.

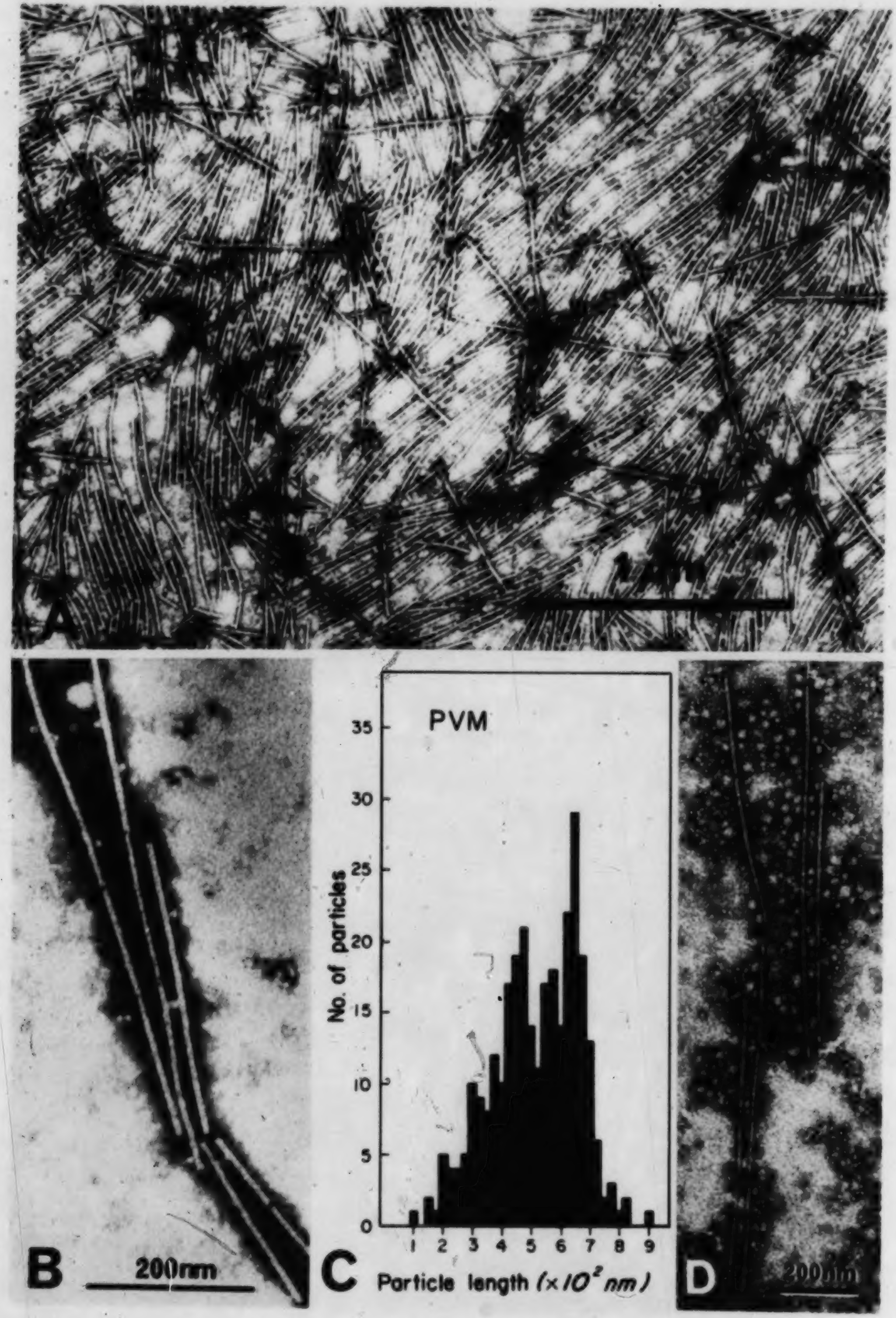
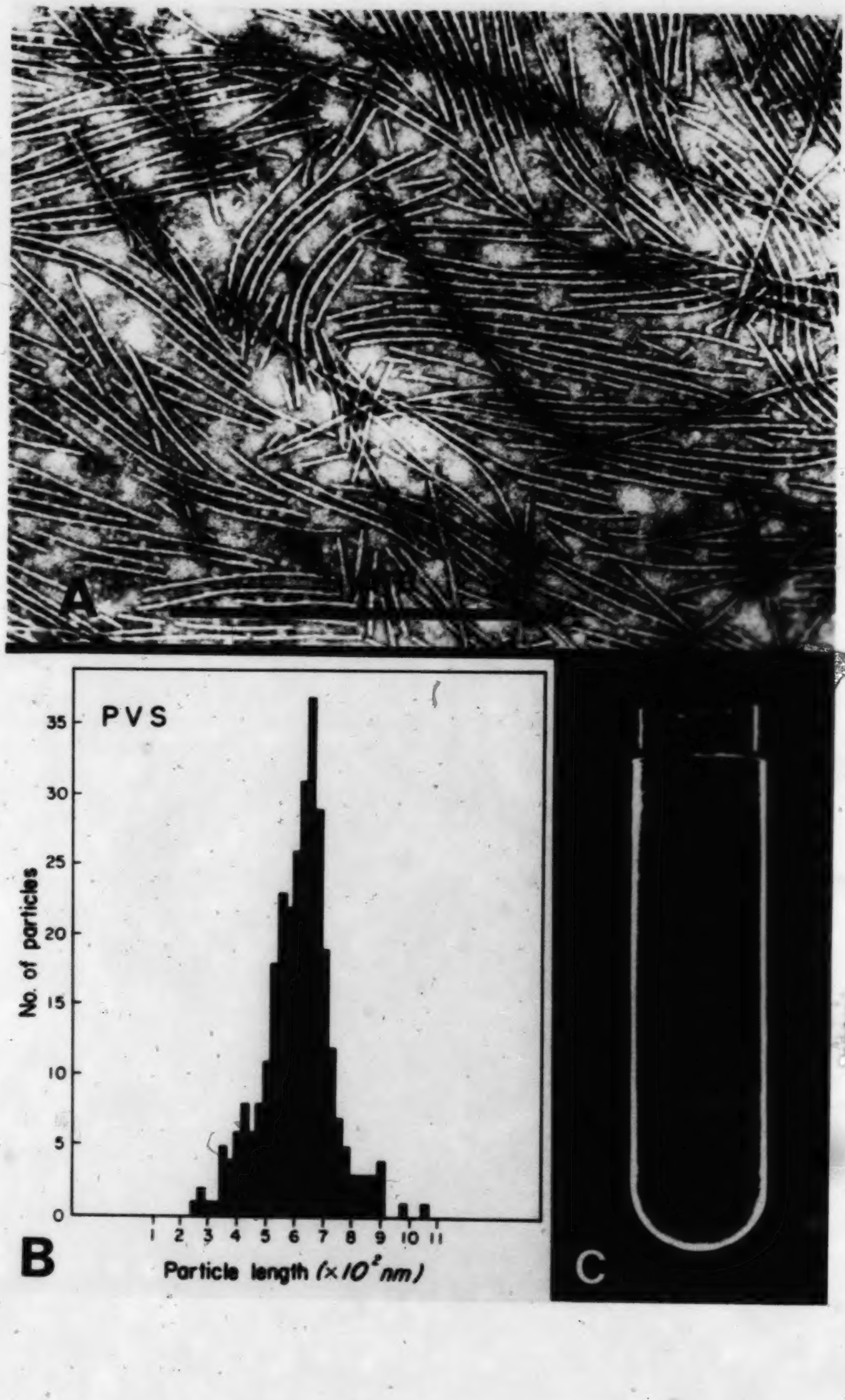

A
B
200nm
C
PVM
No. of particles
0
5
10
15
20
25
30
35
1
2
3
4
5
6
7
8
9
Particle length $(\times 10^2\ nm)$
D
200nm

PLATE 3. Electron micrograph, length-distribution and cesium chloride (CsCl) isopycnic banding of potato virus S (PVS) obtained by the polyethylene glycol-sodium chloride purification method.

A. PVS particles stained with 2% sodium phosphotungstate, pH 7.0.

B. A histogram showing the length-distribution based on a measurement of 300 PVS particles.

C. A single light scattering band obtained after density centrifugation of purified PVS preparation in 20-40% CsCl gradient.


A
PVS
No. of particles
0
5
10
15
20
25
30
35
1
2
3
4
5
6
7
8
9
10
11
Particle length (×10² nm)
B
C

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# APPENDIX

TABLE 16. Lesion counts: The effect of post-inoculation temperature on the local lesion number of potato virus M in 'Red Kidney' bean.

| Expt. | Leaf no. | Temperature (°C)[a] | | | |
|---|---|---|---|---|---|
| | | 17 | 22 | 27 | 32 |
| I | 1 | 52 | 4 | 0 | 0 |
| | 2 | 64 | 7 | 0 | 0 |
| | 3 | 70 | 7 | 0 | 1 |
| | 4 | 52 | 2 | 0 | 0 |
| | 5 | 35 | 10 | 0 | 0 |
| | 6 | 50 | 4 | 0 | 0 |
| | Lesion no/ half-leaf | 26.9 | 2.8 | 0 | 0.1 |
| | Standard error | 2.5 | 0.6 | 0 | 0.1 |
| II | 1 | 24 | 8 | 0 | 0 |
| | 2 | 25 | 3 | 0 | 0 |
| | 3 | 48 | 5 | 0 | 0 |
| | 4 | 39 | 7 | 0 | 0 |
| | 5 | 10 | 13 | 0 | 0 |
| | 6 | 15 | 17 | 2 | 0 |
| | Lesion no./ half-leaf | 13.4 | 4.4 | 0.2 | 0 |
| | Standard error | 2.9 | 1.1 | 0.2 | 0 |

[a] Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

TABLE 17. Lesion counts: Infectivity of potato virus M extracted from frozen infected potato leaves in 'Red Kidney' bean at various time intervals after their incubation at 4° or at 24° C.

| Half-leaf no. | Time *(hr)*[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 0.5 | | 2 | | 8 | | 24 | | 48 | |
| | | | Temperature *(°C)* | | | | | | | | | |
| | | | 4 | 24 | 4 | 24 | 4 | 24 | 4 | 24 | 4 | 24 |
| 1 | 19 | 23 | 25 | 82 | 33 | 32 | 15 | 7 | 13 | 5 | 6 | 0 |
| 2 | 26 | 28 | 12 | 63 | 103 | 49 | 9 | 1 | 10 | 1 | 8 | 0 |
| 3 | 17 | 24 | 5 | 57 | 9 | 16 | 10 | 5 | 4 | 0 | 5 | 0 |
| 4 | 44 | 37 | 21 | 71 | 17 | 12 | 10 | 3 | 1 | 0 | 2 | 0 |
| 5 | 7 | 3 | 12 | 42 | 42 | 30 | 15 | 0 | 4 | 0 | 5 | 0 |
| 6 | 15 | 12 | 8 | 63 | 35 | 21 | 30 | 0 | 1 | 0 | 1 | 0 |
| 7 | 4 | 4 | 21 | 67 | 206 | 67 | 10 | 3 | 11 | 1 | 1 | 0 |
| 8 | 0 | 3 | 7 | 32 | 74 | 33 | 15 | 0 | 5 | 1 | 5 | 0 |
| 9 | 31 | 37 | 1 | 14 | 83 | 28 | 18 | 5 | 2 | 1 | 1 | 0 |
| 10 | 30 | 20 | 3 | 3 | 114 | 26 | 31 | 0 | 3 | 1 | 3 | 0 |
| 11 | 4 | 10 | 3 | 16 | 104 | 36 | 22 | 1 | 2 | 0 | 3 | 0 |
| 12 | 15 | 16 | 3 | 7 | 193 | 84 | 34 | 2 | 4 | 1 | 2 | 0 |
| Mean | 17.9 | | 10.1 | 43.1 | 84.4 | 36.2 | 18.3 | 2.3 | 5.0 | 0.9 | 3.5 | 0 |
| Standard error | 2.5 | | 2.4 | 8.0 | 18.6 | 6.1 | 2.6 | 0.7 | 1.2 | 0.4 | 0.7 | 0 |
| Infectivity index | 1.0 | | 0.6 | 2.4 | 4.7 | 2.0 | 1.0 | 0.1 | 0.3 | 0.1 | 0.2 | 0 |

[a] Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

TABLE 18. Lesion counts: Determination of the infectivity of potato virus M in 'Red Kidney' bean at different growth stages of infected 'King Edward' potato plants.

| Sample[a] | Days after seeding of tubers | Plant height *(cm)* | | Sampling height *(cm)* | | Lesion no./half-leaf | |
|---|---|---|---|---|---|---|---|
| | | *Expt. I* | *Expt. II* | *Expt. I* | *Expt. II* | *Expt. I* | *Expt. II* |
| 1 | 22 | 27.2 | 18.3 | 16.5 | 10.2 | 62.3 | 69.3 |
| 2 | 25 | 35.6 | 27.2 | 20.8 | 19.6 | 15.8 | 22.5 |
| 3 | 28 | 43.2 | 33.0 | 34.3 | 25.4 | 46.5 | 32.0 |
| 4 | 31 | 53.3 | 43.2 | 38.6 | 30.5 | 6.3 | 11.0 |
| 5 | 34 | 59.7 | 50.8 | 50.8 | 36.8 | 68.3 | 46.8 |
| 6 | 37 | 66.8 | 55.9 | 54.6 | 42.4 | 32.3 | 23.3 |
| 7 | 40 | 76.2 | 63.5 | 62.2 | 48.3 | 79.8 | 88.0 |
| 8 | 43[b] | 85.1 | 71.1 | 66.0 | 52.6 | 8.0 | 14.0 |
| 9 | 46 | 92.7 | 80.0 | 74.9 | 61.0 | 24.8 | 20.5 |
| 10 | 49 | 97.3 | 83.8 | 84.6 | 73.7 | 7.8 | 15.3 |

[a] Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

[b] Blossom stage.

TABLE 19A. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) in 'Red Kidney' bean. *Expt. I.*

| Inoculation time | Half-leaf no. | Days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | 5 | | 6 | | 7 | | 9 | |
| | | C[a] | T[b] | C | T | C | T | C | T | C | T |
| 0 time | 1 | 18 | 7 | 41 | 57 | 45 | 262 | 47 | 260 | 47 | 257 |
| | 2 | 14 | 14 | 36 | 84 | 39 | 304 | 39 | 302 | 40 | 300 |
| | 3 | 5 | 3 | 8 | 28 | 9 | 84 | 10 | 91 | 10 | 92 |
| | 4 | 8 | 4 | 12 | 22 | 12 | 82 | 12 | 80 | 12 | 79 |
| | 5 | 10 | 5 | 24 | 51 | 27 | 189 | 29 | 194 | 29 | 196 |
| | 6 | 13 | 18 | 32 | 78 | 35 | 284 | 37 | 308 | 37 | 301 |
| | 7 | 7 | 4 | 11 | 33 | 12 | 60 | 12 | 103 | 12 | 113 |
| | 8 | 6 | 6 | 10 | 34 | 10 | 80 | 12 | 130 | 12 | 133 |
| | 9 | 9 | 10 | 14 | 66 | 14 | 162 | 14 | 166 | 14 | 163 |
| | 10 | 10 | 8 | 17 | 45 | 19 | 117 | 21 | 145 | 21 | 144 |
| | 11 | 8 | 11 | 15 | 68 | 16 | 200 | 16 | 205 | 16 | 207 |
| | 12 | 11 | 6 | 22 | 32 | 24 | 125 | 25 | 151 | 26 | 150 |
| | Mean | 9.9 | 8.0 | 20.2 | 49.8 | 21.8 | 162.4 | 22.8 | 177.9 | 23.0 | 177.9 |
| | Standard error | 1.1 | 1.3 | 3.2 | 6.0 | 3.5 | 24.7 | 3.6 | 22.5 | 3.7 | 21.9 |

[a] C = Control, i.e. 0.33 mg PVM/ml.

[b] T = Test, i.e. 0.33 mg PVM + 0.40 mg PVS/ml.

TABLE 19A. cont.

| Inoculation time (1 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | 5 | | 6 | | 7 | | 9 | |
| | | C | T | C | T | C | T | C | T | C | T |
| M ⟶ S | 1 | 19 | 18 | 34 | 30 | 36 | 50 | 36 | 52 | 36 | 54 |
| | 2 | 24 | 33 | 43 | 55 | 46 | 81 | 50 | 88 | 50 | 86 |
| | 3 | 15 | 15 | 29 | 31 | 30 | 52 | 32 | 57 | 32 | 56 |
| | 4 | 29 | 23 | 37 | 45 | 40 | 57 | 43 | 60 | 43 | 60 |
| | 5 | 31 | 28 | 57 | 41 | 59 | 77 | 62 | 80 | 62 | 82 |
| | 6 | 12 | 25 | 27 | 35 | 28 | 55 | 29 | 54 | 29 | 54 |
| | 7 | 13 | 11 | 25 | 20 | 25 | 35 | 25 | 37 | 25 | 39 |
| | 8 | 16 | 8 | 20 | 17 | 21 | 23 | 23 | 28 | 23 | 27 |
| | 9 | 11 | 10 | 14 | 17 | 15 | 22 | 15 | 24 | 15 | 27 |
| | 10 | 9 | 8 | 17 | 11 | 18 | 18 | 18 | 23 | 18 | 23 |
| | 11 | 10 | 17 | 24 | 27 | 25 | 34 | 27 | 39 | 27 | 37 |
| | 12 | 17 | 19 | 31 | 33 | 33 | 60 | 38 | 61 | 38 | 58 |
| | Mean | 17.2 | 17.9 | 29.8 | 30.2 | 31.3 | 47.0 | 33.2 | 50.3 | 33.2 | 50 |
| | Standard error | 2.1 | 2.3 | 3.4 | 3.7 | 3.6 | 6.0 | 3.9 | 6.0 | 3.9 | 6 |
| S ⟶ M | 1 | 9 | 7 | 16 | 35 | 16 | 48 | 17 | 60 | 17 | 60 |
| | 2 | 6 | 6 | 12 | 29 | 12 | 36 | 12 | 47 | 12 | 46 |
| | 3 | 17 | 9 | 31 | 98 | 33 | 140 | 36 | 150 | 36 | 153 |
| | 4 | 12 | 8 | 26 | 88 | 27 | 160 | 27 | 173 | 27 | 177 |
| | 5 | 5 | 5 | 9 | 25 | 9 | 29 | 9 | 37 | 9 | 36 |
| | 6 | 7 | 5 | 10 | 28 | 10 | 36 | 10 | 34 | 10 | 34 |
| | 7 | 7 | 4 | 11 | 25 | 11 | 35 | 11 | 47 | 11 | 44 |
| | 8 | 8 | 3 | 17 | 34 | 18 | 72 | 20 | 72 | 20 | 69 |
| | 9 | 14 | 4 | 25 | 45 | 26 | 110 | 28 | 131 | 28 | 136 |
| | 10 | 10 | 4 | 20 | 51 | 20 | 84 | 21 | 90 | 21 | 88 |
| | 11 | 6 | 5 | 14 | 39 | 14 | 56 | 15 | 57 | 15 | 58 |
| | 12 | 13 | 2 | 20 | 38 | 21 | 60 | 23 | 71 | 23 | 70 |
| | Mean | 9.5 | 5.2 | 17.6 | 44.6 | 18.1 | 72.2 | 19.1 | 80.1 | 19.1 | 81 |
| | Standard error | 1.1 | 0.6 | 2.1 | 6.9 | 2.2 | 12.5 | 2.4 | 13.3 | 2.4 | 14 |

[a] Second virus was applied 1 hr after.

TABLE 19A. cont.

| Inoculation time (10hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | 5 | | 6 | | 7 | | 9 | |
| | | C | T | C | T | C | T | C | T | C | T |
| M → S | 1 | 11 | 16 | 25 | 22 | 27 | 29 | 29 | 34 | 29 | 34 |
| | 2 | 8 | 5 | 20 | 19 | 21 | 21 | 24 | 28 | 25 | 30 |
| | 3 | 13 | 6 | 24 | 10 | 26 | 11 | 29 | 19 | 29 | 21 |
| | 4 | 9 | 5 | 13 | 22 | 13 | 25 | 15 | 32 | 16 | 34 |
| | 5 | 19 | 16 | 39 | 57 | 43 | 62 | 45 | 73 | 46 | 71 |
| | 6 | 15 | 20 | 30 | 35 | 33 | 50 | 35 | 69 | 36 | 66 |
| | 7 | 15 | 10 | 28 | 21 | 29 | 31 | 30 | 36 | 30 | 39 |
| | 8 | 10 | 13 | 18 | 23 | 18 | 25 | 19 | 31 | 19 | 30 |
| | 9 | 10 | 4 | 19 | 9 | 19 | 17 | 20 | 28 | 20 | 29 |
| | 10 | 9 | 6 | 16 | 10 | 16 | 23 | 17 | 22 | 17 | 23 |
| | 11 | 6 | 6 | 9 | 17 | 10 | 20 | 10 | 23 | 10 | 24 |
| | 12 | 7 | 4 | 11 | 10 | 11 | 15 | 12 | 17 | 12 | 17 |
| | Mean | 11.0 | 9.3 | 21.0 | 21.3 | 22.2 | 27.4 | 23.8 | 34.3 | 24.1 | 34.8 |
| | Standard error | 1.1 | 1.6 | 2.5 | 3.9 | 2.8 | 4.3 | 3.0 | 5.2 | 3.0 | 4.9 |
| S → M | 1 | 14 | 5 | 20 | 21 | 21 | 39 | 21 | 40 | 21 | 40 |
| | 2 | 6 | 13 | 9 | 29 | 11 | 49 | 11 | 57 | 11 | 58 |
| | 3 | 16 | 26 | 26 | 65 | 26 | 94 | 28 | 104 | 30 | 106 |
| | 4 | 22 | 9 | 41 | 34 | 41 | 59 | 44 | 65 | 46 | 64 |
| | 5 | 25 | 17 | 32 | 39 | 33 | 48 | 35 | 54 | 36 | 56 |
| | 6 | 12 | 26 | 23 | 42 | 23 | 50 | 26 | 49 | 27 | 48 |
| | 7 | 19 | 30 | 34 | 88 | 35 | 106 | 37 | 110 | 39 | 108 |
| | 8 | 15 | 20 | 21 | 59 | 21 | 89 | 22 | 88 | 23 | 87 |
| | 9 | 13 | 7 | 24 | 23 | 26 | 31 | 26 | 39 | 26 | 38 |
| | 10 | 9 | 7 | 19 | 30 | 19 | 50 | 20 | 55 | 22 | 57 |
| | 11 | 7 | 8 | 10 | 44 | 11 | 63 | 12 | 67 | 12 | 70 |
| | 12 | 11 | 9 | 18 | 33 | 20 | 64 | 20 | 65 | 20 | 66 |
| | Mean | 14.1 | 14.8 | 23.1 | 42.3 | 23.9 | 61.8 | 25.2 | 66.1 | 26.1 | 66.5 |
| | Standard error | 1.7 | 2.6 | 2.7 | 5.7 | 2.6 | 6.7 | 2.8 | 6.7 | 3.0 | 6.7 |

[a] The second virus was applied 10 hr after.

TABLE. 19B. Lesion counts: Increment curves of local lesions obtained after pre- and post -inoculation treatments with purified potato virus M (PVM) in 'Red Kidney' bean. *Expt. II.*

| Inoculation time | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | 5 | | 6 | | 7 | | 8 | | 9 | |
| | | C[a] | T[b] | C | T | C | T | C | T | C | T | C | T |
| | 1 | 4 | 6 | 18 | 59 | 20 | 84 | 25 | 99 | 25 | 99 | 26 | 99 |
| | 2 | 3 | 8 | 8 | 37 | 14 | 59 | 18 | 65 | 18 | 71 | 18 | 68 |
| | 3 | 4 | 12 | 12 | 64 | 13 | 85 | 23 | 99 | 23 | 108 | 23 | 112 |
| | 4 | 4 | 15 | 19 | 51 | 20 | 78 | 30 | 94 | 30 | 105 | 32 | 108 |
| | 5 | 7 | 20 | 24 | 116 | 27 | 140 | 38 | 171 | 39 | 182 | 39 | 180 |
| | 6 | 13 | 29 | 40 | 111 | 44 | 178 | 55 | 178 | 56 | 188 | 56 | 188 |
| O time | 7 | 0 | 9 | 8 | 23 | 9 | 33 | 12 | 43 | 12 | 50 | 13 | 50 |
| | 8 | 3 | 9 | 11 | 29 | 11 | 40 | 20 | 49 | 20 | 57 | 21 | 60 |
| | 9 | 9 | 18 | 21 | 93 | 21 | 127 | 27 | 145 | 31 | 148 | 31 | 148 |
| | 10 | 10 | 34 | 32 | 109 | 35 | 152 | 40 | 183 | 40 | 200 | 43 | 202 |
| | 11 | 8 | 8 | 19 | 50 | 21 | 64 | 29 | 79 | 29 | 90 | 29 | 90 |
| | 12 | 7 | 9 | 17 | 44 | 17 | 72 | 23 | 92 | 23 | 94 | 23 | 94 |
| | Mean | 6.0 | 14.8 | 19.1 | 65.5 | 21.0 | 92.7 | 28.3 | 108.1 | 28.8 | 116.0 | 29.5 | 117 |
| | Standard error | 1.1 | 2.6 | 2.8 | 9.6 | 2.9 | 13.3 | 3.3 | 14.3 | 3.4 | 14.8 | 3.4 | 15 |

[a] C = Control, i.e. 0.56 mg PVM/ml.

[b] T = Test, i.e. 0.56 mg PVM + 0.80 mg PVS/ml.

TABLE 19B. cont.

| Inoculation time (1 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | 5 | | 6 | | 7 | | 8 | | 9 | |
| | | C | T | C | T | C | T | C | T | C | T | C | T |
| M→S | 1 | 2 | 3 | 9 | 25 | 17 | 37 | 20 | 38 | 20 | 39 | 20 | 40 |
| | 2 | 2 | 4 | 10 | 29 | 19 | 39 | 23 | 46 | 23 | 46 | 23 | 47 |
| | 3 | 5 | 7 | 24 | 70 | 42 | 105 | 45 | 106 | 48 | 106 | 48 | 105 |
| | 4 | 3 | 8 | 14 | 94 | 23 | 134 | 26 | 134 | 34 | 135 | 34 | 135 |
| | 5 | 1 | 3 | 6 | 29 | 14 | 39 | 18 | 46 | 20 | 49 | 20 | 50 |
| | 6 | 8 | 3 | 20 | 47 | 31 | 61 | 33 | 67 | 33 | 71 | 32 | 70 |
| | 7 | 3 | 3 | 10 | 47 | 21 | 64 | 25 | 69 | 25 | 74 | 25 | 73 |
| | 8 | 7 | 1 | 19 | 36 | 30 | 66 | 34 | 68 | 34 | 67 | 33 | 66 |
| | 9 | 3 | 8 | 11 | 81 | 22 | 136 | 24 | 153 | 24 | 160 | 24 | 160 |
| | 10 | 2 | 7 | 9 | 72 | 21 | 107 | 25 | 115 | 25 | 122 | 25 | 122 |
| | 11 | 1 | 1 | 5 | 20 | 13 | 37 | 15 | 41 | 15 | 46 | 15 | 44 |
| | 12 | 1 | 2 | 4 | 19 | 10 | 37 | 12 | 46 | 12 | 47 | 12 | 45 |
| | Mean | 3.2 | 4.2 | 11.8 | 47.4 | 21.9 | 71.8 | 25.0 | 77.4 | 26.1 | 80.2 | 25.9 | 79.8 |
| | Standard error | 0.7 | 0.8 | 1.8 | 7.4 | 2.6 | 11.1 | 2.6 | 11.4 | 2.8 | 11.7 | 2.8 | 11.8 |
| S→M | 1 | 8 | 11 | 20 | 52 | 31 | 89 | 40 | 99 | 42 | 107 | 43 | 112 |
| | 2 | 3 | 9 | 14 | 86 | 20 | 142 | 30 | 148 | 31 | 151 | 32 | 150 |
| | 3 | 1 | 10 | 5 | 79 | 7 | 115 | 9 | 121 | 13 | 124 | 13 | 120 |
| | 4 | 3 | 11 | 6 | 62 | 10 | 73 | 14 | 87 | 17 | 97 | 17 | 99 |
| | 5 | 0 | 7 | 5 | 35 | 9 | 42 | 11 | 52 | 11 | 53 | 11 | 52 |
| | 6 | 3 | 8 | 7 | 25 | 11 | 28 | 13 | 40 | 13 | 41 | 13 | 42 |
| | 7 | 6 | 13 | 22 | 61 | 26 | 87 | 33 | 89 | 36 | 95 | 36 | 94 |
| | 8 | 2 | 16 | 13 | 88 | 18 | 105 | 23 | 116 | 25 | 125 | 25 | 120 |
| | 9 | 1 | 13 | 5 | 60 | 8 | 73 | 10 | 87 | 15 | 93 | 15 | 93 |
| | 10 | 3 | 9 | 11 | 44 | 13 | 46 | 20 | 58 | 23 | 60 | 23 | 60 |
| | 11 | 4 | 8 | 12 | 44 | 19 | 45 | 24 | 46 | 24 | 49 | 24 | 49 |
| | 12 | 2 | 6 | 8 | 43 | 14 | 44 | 21 | 55 | 21 | 55 | 21 | 54 |
| | Mean | 3.0 | 10.1 | 10.7 | 56.6 | 15.5 | 74.1 | 20.7 | 83.2 | 22.6 | 87.5 | 22.8 | 87.1 |
| | Standard error | 0.6 | 0.8 | 1.7 | 5.8 | 2.2 | 10.1 | 2.9 | 9.8 | 2.8 | 10.3 | 2.9 | 10.1 |

[a] The second virus was applied 1 hr after.

TABLE 19B. cont.

| Inoculation time (5 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | 5 | | 6 | | 7 | | 8 | | 9 | |
| | | C | T | C | T | C | T | C | T | C | T | C | T |
| M → S | 1 | 12 | 16 | 29 | 30 | 32 | 53 | 34 | 54 | 35 | 53 | 36 | 52 |
| | 2 | 8 | 12 | 13 | 32 | 15 | 43 | 18 | 44 | 20 | 44 | 20 | 42 |
| | 3 | 3 | 7 | 5 | 10 | 7 | 18 | 11 | 21 | 11 | 23 | 11 | 24 |
| | 4 | 3 | 11 | 7 | 19 | 10 | 38 | 13 | 39 | 14 | 39 | 14 | 39 |
| | 5 | 4 | 9 | 6 | 18 | 9 | 22 | 10 | 24 | 10 | 24 | 10 | 24 |
| | 6 | 2 | 10 | 4 | 12 | 7 | 15 | 8 | 18 | 8 | 17 | 8 | 22 |
| | 7 | 8 | 17 | 20 | 39 | 24 | 49 | 27 | 50 | 27 | 54 | 27 | 52 |
| | 8 | 9 | 15 | 12 | 18 | 14 | 31 | 16 | 39 | 16 | 40 | 16 | 40 |
| | 9 | 3 | 10 | 7 | 14 | 9 | 21 | 9 | 25 | 9 | 23 | 9 | 22 |
| | 10 | 4 | 9 | 8 | 14 | 11 | 20 | 14 | 19 | 14 | 21 | 14 | 23 |
| | 11 | 11 | 20 | 26 | 36 | 29 | 51 | 31 | 53 | 31 | 57 | 31 | 57 |
| | 12 | 7 | 7 | 14 | 26 | 18 | 42 | 21 | 45 | 21 | 45 | 21 | 44 |
| | Mean | 6.2 | 11.9 | 12.6 | 22.3 | 15.4 | 33.6 | 17.7 | 35.9 | 18.0 | 36.7 | 18.1 | 37 |
| | Standard error | 1.0 | 1.2 | 2.4 | 2.9 | 2.5 | 4.1 | 2.5 | 4.0 | 2.6 | 4.2 | 2.6 | 4 |
| S → M | 1 | 4 | 7 | 15 | 49 | 31 | 72 | 35 | 85 | 35 | 93 | 35 | 97 |
| | 2 | 2 | 3 | 10 | 45 | 16 | 67 | 18 | 74 | 21 | 78 | 23 | 78 |
| | 3 | 3 | 5 | 8 | 32 | 11 | 56 | 14 | 60 | 16 | 63 | 18 | 62 |
| | 4 | 6 | 6 | 14 | 50 | 29 | 78 | 29 | 78 | 29 | 78 | 29 | 78 |
| | 5 | 1 | 2 | 9 | 16 | 11 | 25 | 12 | 25 | 12 | 27 | 13 | 27 |
| | 6 | 2 | 4 | 12 | 14 | 20 | 22 | 22 | 28 | 22 | 34 | 22 | 33 |
| | 7 | 5 | 5 | 20 | 40 | 41 | 72 | 42 | 77 | 43 | 88 | 43 | 89 |
| | 8 | 7 | 10 | 23 | 75 | 39 | 94 | 43 | 105 | 45 | 114 | 45 | 113 |
| | 9 | 3 | 2 | 10 | 19 | 18 | 28 | 18 | 35 | 18 | 36 | 18 | 35 |
| | 10 | 1 | 3 | 7 | 12 | 9 | 24 | 9 | 27 | 10 | 31 | 10 | 32 |
| | 11 | 0 | 2 | 6 | 15 | 10 | 19 | 10 | 20 | 10 | 23 | 10 | 22 |
| | 12 | 1 | 5 | 5 | 16 | 8 | 20 | 9 | 25 | 9 | 28 | 9 | 29 |
| | Mean | 2.9 | 4.5 | 11.6 | 31.9 | 20.3 | 48.1 | 21.8 | 53.3 | 22.5 | 57.8 | 22.9 | 58 |
| | Standard error | 0.6 | 0.7 | 1.6 | 5.8 | 3.4 | 8.0 | 3.6 | 8.6 | 3.7 | 9.1 | 3.6 | 9 |

[a] The second virus was applied 5 hr after.

TABLE 19B. cont.

| Inoculation time (10 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | 5 | | 6 | | 7 | | 8 | | 9 | |
| | | C | T | C | T | C | T | C | T | C | T | C | T |
| M → S | 1 | 3 | 3 | 8 | 16 | 11 | 20 | 15 | 24 | 15 | 25 | 15 | 25 |
| | 2 | 1 | 6 | 7 | 13 | 10 | 18 | 10 | 26 | 12 | 26 | 12 | 26 |
| | 3 | 3 | 10 | 12 | 20 | 16 | 36 | 20 | 49 | 25 | 53 | 26 | 53 |
| | 4 | 9 | 8 | 15 | 32 | 20 | 47 | 23 | 50 | 32 | 52 | 32 | 52 |
| | 5 | 3 | 1 | 5 | 4 | 8 | 11 | 8 | 12 | 9 | 12 | 9 | 12 |
| | 6 | 1 | 3 | 4 | 7 | 6 | 11 | 6 | 14 | 6 | 16 | 6 | 16 |
| | 7 | 4 | 4 | 9 | 15 | 15 | 20 | 16 | 23 | 18 | 28 | 20 | 28 |
| | 8 | 4 | 1 | 10 | 8 | 12 | 17 | 12 | 17 | 12 | 21 | 12 | 21 |
| | 9 | 4 | 1 | 7 | 8 | 9 | 13 | 9 | 18 | 11 | 22 | 11 | 21 |
| | 10 | 1 | 3 | 5 | 8 | 7 | 13 | 9 | 17 | 10 | 17 | 10 | 17 |
| | 11 | 3 | 6 | 10 | 15 | 12 | 24 | 13 | 26 | 15 | 27 | 15 | 26 |
| | 12 | 5 | 5 | 14 | 12 | 17 | 22 | 19 | 27 | 23 | 27 | 23 | 27 |
| | Mean | 3.4 | 4.3 | 8.8 | 13.2 | 11.9 | 21.0 | 13.3 | 25.3 | 15.7 | 27.2 | 15.9 | 27 |
| | Standard error | 0.6 | 2.9 | 1.0 | 2.2 | 1.3 | 3.1 | 1.5 | 3.6 | 2.2 | 3.7 | 2.2 | 4 |
| S → M | 1 | 4 | 2 | 20 | 25 | 22 | 40 | 22 | 56 | 24 | 62 | 24 | 61 |
| | 2 | 5 | 3 | 20 | 20 | 20 | 36 | 20 | 36 | 21 | 41 | 21 | 41 |
| | 3 | 2 | 1 | 11 | 12 | 12 | 19 | 12 | 26 | 12 | 26 | 12 | 26 |
| | 4 | 2 | 0 | 8 | 7 | 9 | 16 | 9 | 24 | 10 | 27 | 10 | 27 |
| | 5 | 2 | 3 | 14 | 32 | 15 | 45 | 15 | 56 | 17 | 57 | 17 | 57 |
| | 6 | 3 | 2 | 19 | 27 | 20 | 30 | 20 | 42 | 21 | 43 | 21 | 43 |
| | 7 | 0 | 1 | 6 | 9 | 7 | 18 | 7 | 21 | 7 | 24 | 7 | 24 |
| | 8 | 2 | 0 | 9 | 8 | 9 | 15 | 9 | 17 | 9 | 17 | 9 | 17 |
| | 9 | 2 | 1 | 18 | 17 | 21 | 24 | 21 | 34 | 23 | 43 | 23 | 43 |
| | 10 | 6 | 2 | 24 | 30 | 30 | 57 | 30 | 64 | 34 | 64 | 34 | 63 |
| | 11 | 2 | 0 | 10 | 5 | 12 | 15 | 12 | 21 | 14 | 21 | 14 | 21 |
| | 12 | 2 | 1 | 7 | 15 | 7 | 26 | 8 | 29 | 9 | 29 | 10 | 29 |
| | Mean | 2.7 | 1.3 | 13.8 | 17.3 | 15.3 | 28.4 | 15.4 | 35.5 | 16.8 | 37.8 | 16.8 | 38 |
| | Standard error | 0.5 | 0.3 | 1.8 | 2.7 | 2.1 | 3.9 | 2.1 | 4.5 | 2.3 | 4.7 | 2.3 | 5 |

[a] The second virus was applied 10 hr after.

TABLE 20A. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with potato virus S(PVS) in *Chenopodium quinoa*. *Expt. I.*

| Inoculation time | Half-leaf no. | Days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 10 | |
| | | C[a] | T[b] | C | T | C | T | C | T | C | T |
| O time | 1 | 0 | 1 | 3 | 11 | 11 | 29 | 30 | 80 | 30 | 79 |
| | 2 | 0 | 1 | 4 | 15 | 19 | 43 | 38 | 155 | 39 | 155 |
| | 3 | 2 | 4 | 11 | 38 | 21 | 118 | 58 | 277 | 60 | 270 |
| | 4 | 2 | 3 | 9 | 31 | 32 | 114 | 79 | 222 | 81 | 227 |
| | 5 | 0 | 0 | 4 | 11 | 8 | 39 | 21 | 88 | 21 | 89 |
| | 6 | 0 | 1 | 5 | 14 | 14 | 42 | 33 | 107 | 33 | 111 |
| | 7 | 0 | 3 | 7 | 18 | 14 | 44 | 30 | 153 | 30 | 160 |
| | 8 | 1 | 5 | 7 | 39 | 20 | 95 | 71 | 201 | 71 | 199 |
| | 9 | 0 | 3 | 3 | 22 | 9 | 45 | 29 | 99 | 29 | 98 |
| | 10 | 0 | 4 | 5 | 33 | 31 | 82 | 77 | 197 | 79 | 195 |
| | 11 | 3 | 7 | 12 | 60 | 34 | 135 | 106 | 284 | 105 | 280 |
| | 12 | 1 | 3 | 5 | 31 | 29 | 63 | 50 | 131 | 52 | 133 |
| | Mean | 0.8 | 2.9 | 6.3 | 26.9 | 20.2 | 70.8 | 51.8 | 166.2 | 52.5 | 166.3 |
| | Standard error | 0.3 | 0.6 | 0.9 | 4.2 | 2.7 | 10.6 | 7.7 | 20.3 | 7.7 | 19.8 |

[a] C=Control, i.e. 0.08 mg PVS/ml.

[b] T=Test, i.e. 0.08 mg PVS + 1.67 mg PVM/ml.

TABLE 20A cont.

| Inoculation time (1 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 10 | |
| | | C | T | C | T | C | T | C | T | C | T |
| S → M | 1 | 1 | 1 | 6 | 12 | 18 | 37 | 31 | 89 | 31 | 88 |
| | 2 | 0 | 3 | 9 | 13 | 25 | 43 | 47 | 89 | 49 | 90 |
| | 3 | 1 | 1 | 8 | 11 | 28 | 46 | 54 | 100 | 56 | 101 |
| | 4 | 4 | 5 | 23 | 22 | 43 | 91 | 103 | 178 | 104 | 175 |
| | 5 | 0 | 0 | 5 | 7 | 10 | 20 | 20 | 44 | 22 | 48 |
| | 6 | 0 | 2 | 5 | 13 | 10 | 32 | 22 | 54 | 24 | 57 |
| | 7 | 0 | 1 | 4 | 10 | 16 | 23 | 29 | 68 | 30 | 68 |
| | 8 | 1 | 4 | 11 | 16 | 22 | 45 | 34 | 90 | 34 | 89 |
| | 9 | 0 | 3 | 7 | 11 | 12 | 33 | 25 | 85 | 25 | 87 |
| | 10 | 2 | 8 | 12 | 45 | 29 | 84 | 58 | 150 | 58 | 147 |
| | 11 | 1 | 4 | 12 | 22 | 31 | 65 | 69 | 115 | 70 | 114 |
| | 12 | 3 | 10 | 19 | 51 | 40 | 94 | 99 | 203 | 100 | 200 |
| | Mean | 1.1 | 3.5 | 10.1 | 19.4 | 23.7 | 51.1 | 49.3 | 105.4 | 50.3 | 105 |
| | Standard error | 0.4 | 0.9 | 1.7 | 4.1 | 3.2 | 7.5 | 8.3 | 14.0 | 8.3 | 13 |
| M → S | 1 | 0 | 2 | 2 | 13 | 7 | 39 | 17 | 56 | 18 | 58 |
| | 2 | 1 | 0 | 2 | 9 | 8 | 28 | 18 | 70 | 18 | 71 |
| | 3 | 0 | 4 | 3 | 15 | 11 | 31 | 30 | 89 | 30 | 88 |
| | 4 | 0 | 4 | 2 | 17 | 10 | 38 | 28 | 71 | 28 | 70 |
| | 5 | 0 | 2 | 2 | 11 | 8 | 31 | 15 | 62 | 16 | 62 |
| | 6 | 1 | 6 | 3 | 20 | 12 | 50 | 34 | 103 | 34 | 101 |
| | 7 | 1 | 6 | 10 | 28 | 27 | 55 | 60 | 116 | 60 | 114 |
| | 8 | 2 | 18 | 7 | 58 | 17 | 90 | 40 | 192 | 40 | 190 |
| | 9 | 1 | 4 | 4 | 22 | 24 | 50 | 50 | 100 | 50 | 99 |
| | 10 | 2 | 8 | 6 | 36 | 21 | 70 | 46 | 154 | 46 | 154 |
| | 11 | 2 | 10 | 8 | 38 | 31 | 82 | 75 | 224 | 75 | 221 |
| | 12 | 2 | 15 | 10 | 67 | 30 | 137 | 68 | 248 | 70 | 245 |
| | Mean | 1.0 | 6.6 | 4.9 | 27.8 | 17.2 | 58.4 | 40.1 | 123.8 | 40.4 | 123 |
| | Standard error | 0.3 | 1.6 | 0.9 | 5.4 | 2.6 | 9.2 | 5.8 | 19.0 | 5.8 | 19 |

[a] The second virus was applied 1 hr after.

TABLE 20A. cont.

| Inoculation time (10 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 10 | |
| | | C | T | C | T | C | T | C | T | C | T |
| S ⟶ M | 1 | 0 | 0 | 0 | 3 | 5 | 10 | 15 | 20 | 15 | 20 |
| | 2 | 0 | 0 | 1 | 4 | 7 | 13 | 18 | 35 | 18 | 36 |
| | 3 | 0 | 0 | 0 | 6 | 7 | 13 | 21 | 31 | 22 | 30 |
| | 4 | 2 | 2 | 5 | 10 | 15 | 30 | 58 | 80 | 58 | 79 |
| | 5 | 0 | 0 | 0 | 2 | 6 | 7 | 16 | 18 | 16 | 19 |
| | 6 | 1 | 0 | 7 | 7 | 19 | 18 | 52 | 57 | 52 | 60 |
| | 7 | 2 | 1 | 10 | 13 | 29 | 45 | 75 | 102 | 75 | 100 |
| | 8 | 0 | 0 | 4 | 11 | 17 | 35 | 62 | 77 | 61 | 74 |
| | 9 | 0 | 0 | 0 | 2 | 6 | 9 | 20 | 20 | 20 | 24 |
| | 10 | 0 | 0 | 1 | 4 | 8 | 10 | 23 | 30 | 23 | 30 |
| | 11 | 1 | 1 | 4 | 3 | 13 | 13 | 36 | 33 | 36 | 33 |
| | 12 | 0 | 0 | 2 | 6 | 19 | 15 | 46 | 53 | 46 | 52 |
| | Mean | 0.5 | 0.3 | 2.8 | 5.9 | 12.6 | 18.2 | 36.8 | 46.4 | 36.8 | 46 |
| | Standard error | 0.2 | 0.2 | 0.9 | 1.1 | 2.1 | 3.5 | 6.1 | 7.9 | 6.0 | 8 |
| M ⟶ S | 1 | 0 | 0 | 1 | 9 | 4 | 26 | 13 | 40 | 13 | 45 |
| | 2 | 0 | 0 | 0 | 10 | 5 | 32 | 15 | 64 | 16 | 65 |
| | 3 | 0 | 0 | 2 | 10 | 10 | 41 | 20 | 93 | 23 | 92 |
| | 4 | 1 | 1 | 6 | 18 | 18 | 67 | 60 | 134 | 60 | 131 |
| | 5 | 0 | 0 | 1 | 7 | 5 | 33 | 14 | 64 | 14 | 68 |
| | 6 | 0 | 0 | 2 | 5 | 7 | 33 | 17 | 73 | 17 | 73 |
| | 7 | 0 | 1 | 4 | 16 | 15 | 64 | 32 | 140 | 34 | 137 |
| | 8 | 1 | 1 | 4 | 20 | 17 | 73 | 45 | 150 | 45 | 148 |
| | 9 | 0 | 0 | 1 | 10 | 7 | 39 | 16 | 77 | 16 | 76 |
| | 10 | 0 | 0 | 1 | 5 | 6 | 20 | 23 | 60 | 23 | 60 |
| | 11 | 1 | 2 | 5 | 32 | 20 | 100 | 54 | 168 | 54 | 163 |
| | 12 | 0 | 0 | 1 | 8 | 7 | 31 | 25 | 70 | 25 | 72 |
| | Mean | 0.3 | 0.4 | 2.3 | 12.5 | 10.1 | 46.6 | 27.8 | 94.4 | 28.3 | 94 |
| | Standard error | 0.1 | 0.2 | 0.6 | 2.3 | 1.7 | 6.9 | 4.7 | 12.2 | 4.7 | 11 |

[a] The second virus was applied 10 hr. after.

TABLE 20B. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with potato virus S (PVS) in *Chenopodium quinoa*. *Expt. II.*

| Inoculation time | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 10 | | 11 | |
| | | C[a] | T[b] | C | T | C | T | C | T | C | T | C | T |
| 0 time | 1 | 2 | 7 | 4 | 17 | 14 | 51 | 37 | 106 | 57 | 168 | 57 | 165 |
| | 2 | 0 | 8 | 2 | 19 | 8 | 66 | 20 | 150 | 37 | 231 | 37 | 227 |
| | 3 | 0 | 1 | 0 | 6 | 7 | 17 | 16 | 52 | 30 | 99 | 30 | 100 |
| | 4 | 2 | 0 | 3 | 4 | 6 | 15 | 13 | 40 | 27 | 73 | 27 | 78 |
| | 5 | 1 | 3 | 3 | 9 | 10 | 33 | 27 | 92 | 41 | 148 | 41 | 143 |
| | 6 | 1 | 2 | 2 | 7 | 9 | 30 | 19 | 73 | 34 | 109 | 34 | 106 |
| | 7 | 1 | 2 | 3 | 5 | 7 | 22 | 15 | 63 | 28 | 84 | 28 | 84 |
| | 8 | 0 | 1 | 1 | 5 | 5 | 15 | 9 | 46 | 19 | 73 | 19 | 76 |
| | 9 | 2 | 5 | 3 | 14 | 15 | 45 | 33 | 107 | 53 | 178 | 53 | 175 |
| | 10 | 3 | 4 | 4 | 14 | 20 | 60 | 47 | 116 | 79 | 225 | 78 | 222 |
| | 11 | 0 | 2 | 1 | 6 | 6 | 16 | 15 | 48 | 22 | 89 | 22 | 88 |
| | 12 | 0 | 2 | 1 | 4 | 4 | 14 | 12 | 25 | 20 | 55 | 20 | 57 |
| | Mean | 1.0 | 3.1 | 2.3 | 9.2 | 9.3 | 32.0 | 22.0 | 76.5 | 37.3 | 127.7 | 37.2 | 127 |
| | Standard error | 0.3 | 0.7 | 0.4 | 1.6 | 1.4 | 5.5 | 3.4 | 10.9 | 5.2 | 17.5 | 5.1 | 17 |

[a] C = Control, i.e. 0.16 mg PVS/ml.

[b] T = Test, i.e. 0.16 mg PVS + 2.78 mg PVM/ml.

TABLE 20B. cont.

| Inoculation time (1 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 10 | | 11 | |
| | | C | T | C | T | C | T | C | T | C | T | C | T |
| S → M | 1 | 0 | 2 | 1 | 7 | 6 | 13 | 13 | 25 | 26 | 38 | 26 | 40 |
| | 2 | 0 | 3 | 2 | 9 | 8 | 16 | 19 | 31 | 28 | 48 | 28 | 51 |
| | 3 | 0 | 0 | 1 | 7 | 5 | 21 | 11 | 49 | 24 | 64 | 24 | 65 |
| | 4 | 1 | 0 | 3 | 4 | 9 | 13 | 21 | 30 | 32 | 41 | 32 | 40 |
| | 5 | 3 | 9 | 8 | 23 | 21 | 60 | 47 | 130 | 77 | 170 | 79 | 167 |
| | 6 | 2 | 12 | 6 | 28 | 14 | 45 | 31 | 110 | 64 | 133 | 67 | 130 |
| | 7 | 2 | 5 | 5 | 12 | 10 | 29 | 27 | 70 | 38 | 82 | 38 | 80 |
| | 8 | 1 | 4 | 4 | 10 | 19 | 37 | 30 | 72 | 45 | 108 | 47 | 106 |
| | 9 | 3 | 3 | 5 | 11 | 17 | 40 | 32 | 95 | 50 | 106 | 53 | 110 |
| | 10 | 2 | 2 | 3 | 6 | 14 | 31 | 27 | 77 | 36 | 89 | 37 | 87 |
| | 11 | 0 | 1 | 2 | 5 | 8 | 14 | 15 | 30 | 19 | 40 | 19 | 44 |
| | 12 | 1 | 3 | 3 | 8 | 10 | 19 | 20 | 40 | 30 | 59 | 30 | 60 |
| | Mean | 1.3 | 3.7 | 3.6 | 10.8 | 11.8 | 28.2 | 24.4 | 63.3 | 39.1 | 81.5 | 40.0 | 82 |
| | Standard error | 0.3 | 1.0 | 0.6 | 2.1 | 1.5 | 4.3 | 2.9 | 10.1 | 5.0 | 12.0 | 5.3 | 12 |
| M → S | 1 | 0 | 0 | 0 | 3 | 4 | 14 | 13 | 36 | 22 | 87 | 22 | 86 |
| | 2 | 0 | 0 | 0 | 5 | 4 | 28 | 12 | 53 | 29 | 121 | 31 | 119 |
| | 3 | 0 | 2 | 3 | 18 | 8 | 39 | 28 | 89 | 54 | 209 | 55 | 204 |
| | 4 | 1 | 1 | 4 | 10 | 11 | 33 | 32 | 95 | 60 | 196 | 62 | 193 |
| | 5 | 0 | 0 | 1 | 9 | 6 | 27 | 15 | 49 | 25 | 82 | 26 | 83 |
| | 6 | 0 | 1 | 2 | 12 | 7 | 25 | 17 | 48 | 31 | 96 | 31 | 94 |
| | 7 | 0 | 1 | 2 | 11 | 7 | 21 | 15 | 44 | 21 | 63 | 22 | 65 |
| | 8 | 0 | 0 | 0 | 4 | 7 | 13 | 17 | 31 | 20 | 79 | 21 | 81 |
| | 9 | 0 | 0 | 0 | 8 | 8 | 20 | 20 | 47 | 38 | 77 | 38 | 80 |
| | 10 | 0 | 1 | 1 | 7 | 13 | 24 | 33 | 67 | 43 | 157 | 43 | 155 |
| | 11 | 0 | 0 | 1 | 4 | 4 | 11 | 9 | 29 | 14 | 51 | 14 | 54 |
| | 12 | 0 | 0 | 0 | 4 | 3 | 10 | 8 | 28 | 15 | 42 | 15 | 45 |
| | Mean | 0.1 | 0.5 | 1.2 | 7.9 | 6.8 | 22.1 | 18.3 | 51.3 | 31.0 | 105.0 | 31.7 | 105 |
| | Standard error | 0.1 | 0.2 | 0.4 | 1.3 | 0.9 | 2.6 | 2.4 | 6.4 | 4.3 | 15.8 | 4.4 | 15 |

[a] The second virus was applied 1 hr after.

TABLE 20B. cont.

| Inoculation time (5 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 10 | | 11 | |
| | | C | T | C | T | C | T | C | T | C | T | C | T |
| | 1 | 3 | 1 | 5 | 2 | 10 | 30 | 21 | 86 | 54 | 98 | 53 | 95 |
| | 2 | 0 | 0 | 0 | 0 | 5 | 20 | 11 | 50 | 29 | 67 | 29 | 68 |
| | 3 | 0 | 0 | 1 | 0 | 3 | 10 | 7 | 40 | 27 | 69 | 27 | 67 |
| | 4 | 1 | 0 | 3 | 1 | 8 | 21 | 14 | 66 | 44 | 88 | 44 | 86 |
| | 5 | 0 | 0 | 1 | 0 | 4 | 15 | 10 | 45 | 30 | 70 | 30 | 70 |
| | 6 | 0 | 1 | 1 | 2 | 3 | 20 | 7 | 60 | 24 | 67 | 24 | 66 |
| S → M | 7 | 0 | 0 | 1 | 1 | 3 | 14 | 8 | 30 | 16 | 40 | 16 | 42 |
| | 8 | 0 | 0 | 0 | 0 | 2 | 7 | 5 | 21 | 14 | 31 | 14 | 31 |
| | 9 | 0 | 0 | 1 | 1 | 6 | 10 | 13 | 40 | 41 | 75 | 41 | 76 |
| | 10 | 0 | 0 | 0 | 0 | 2 | 8 | 5 | 35 | 20 | 50 | 20 | 51 |
| | 11 | 1 | 0 | 3 | 2 | 8 | 24 | 17 | 72 | 60 | 88 | 60 | 89 |
| | 12 | 2 | 1 | 5 | 3 | 12 | 31 | 25 | 103 | 77 | 111 | 75 | 110 |
| | Mean | 0.6 | 0.3 | 1.7 | 1.0 | 5.5 | 17.5 | 11.9 | 54.0 | 36.3 | 71.2 | 36.1 | 71 |
| | Standard error | 0.3 | 0.1 | 0.5 | 0.3 | 1.0 | 2.4 | 1.8 | 7.0 | 5.6 | 6.7 | 5.5 | 7 |
| | 1 | 1 | 0 | 2 | 0 | 4 | 13 | 11 | 53 | 21 | 63 | 21 | 64 |
| | 2 | 0 | 1 | 1 | 2 | 4 | 22 | 12 | 69 | 30 | 90 | 30 | 88 |
| | 3 | 0 | 2 | 0 | 4 | 3 | 12 | 9 | 49 | 15 | 60 | 15 | 60 |
| | 4 | 2 | 0 | 3 | 1 | 6 | 15 | 10 | 58 | 27 | 69 | 27 | 70 |
| | 5 | 2 | 3 | 4 | 5 | 12 | 35 | 27 | 91 | 50 | 123 | 50 | 120 |
| | 6 | 1 | 1 | 2 | 3 | 10 | 40 | 24 | 111 | 61 | 111 | 60 | 110 |
| M → S | 7 | 1 | 1 | 2 | 2 | 6 | 23 | 14 | 69 | 39 | 82 | 39 | 81 |
| | 8 | 0 | 0 | 1 | 1 | 5 | 23 | 14 | 67 | 32 | 74 | 32 | 75 |
| | 9 | 0 | 0 | 0 | 1 | 3 | 14 | 8 | 47 | 16 | 58 | 16 | 60 |
| | 10 | 2 | 1 | 3 | 2 | 9 | 30 | 17 | 87 | 42 | 100 | 42 | 101 |
| | 11 | 1 | 0 | 2 | 1 | 5 | 19 | 10 | 59 | 14 | 63 | 14 | 63 |
| | 12 | 0 | 0 | 0 | 0 | 2 | 11 | 6 | 32 | 10 | 47 | 10 | 48 |
| | Mean | 0.8 | 0.8 | 1.7 | 1.8 | 5.8 | 21.4 | 13.5 | 66.0 | 29.8 | 78.3 | 29.7 | 78 |
| | Standard error | 0.2 | 0.3 | 0.4 | 0.4 | 0.9 | 2.7 | 1.8 | 6.3 | 4.6 | 6.7 | 4.5 | 6 |

[a] The second virus was applied 5 hr after.

TABLE 20B. cont.

| Inoculation time (10 hr)[a] | Half-leaf no. | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 10 | | 11 | |
| | | C | T | C | T | C | T | C | T | C | T | C | T |
| S → M | 1 | 0 | 0 | 2 | 10 | 7 | 22 | 13 | 41 | 32 | 70 | 32 | 69 |
| | 2 | 0 | 0 | 1 | 4 | 3 | 10 | 8 | 23 | 27 | 37 | 27 | 40 |
| | 3 | 1 | 0 | 2 | 3 | 4 | 18 | 7 | 33 | 17 | 48 | 19 | 47 |
| | 4 | 0 | 0 | 0 | 7 | 4 | 17 | 10 | 30 | 27 | 55 | 27 | 58 |
| | 5 | 0 | 1 | 4 | 19 | 9 | 34 | 27 | 71 | 42 | 88 | 42 | 85 |
| | 6 | 1 | 0 | 3 | 19 | 7 | 40 | 17 | 91 | 50 | 131 | 50 | 128 |
| | 7 | 0 | 0 | 0 | 2 | 2 | 9 | 4 | 16 | 14 | 29 | 14 | 30 |
| | 8 | 0 | 0 | 0 | 2 | 2 | 9 | 4 | 18 | 13 | 32 | 13 | 32 |
| | 9 | 0 | 0 | 0 | 7 | 3 | 12 | 9 | 23 | 16 | 37 | 19 | 37 |
| | 10 | 0 | 0 | 0 | 5 | 3 | 13 | 7 | 26 | 19 | 48 | 19 | 48 |
| | 11 | 1 | 0 | 2 | 6 | 5 | 20 | 11 | 41 | 32 | 66 | 32 | 64 |
| | 12 | 0 | 0 | 0 | 12 | 6 | 25 | 13 | 46 | 30 | 73 | 30 | 74 |
| | Mean | 0.3 | 0.1 | 1.2 | 8.0 | 4.6 | 19.1 | 10.8 | 38.3 | 26.6 | 59.5 | 27.0 | 59 |
| | Standard error | 0.1 | 0.1 | 0.4 | 1.7 | 0.7 | 2.9 | 1.8 | 6.5 | 3.3 | 8.4 | 3.2 | 8 |
| M → S | 1 | 0 | 0 | 1 | 2 | 7 | 15 | 13 | 50 | 25 | 66 | 25 | 66 |
| | 2 | 0 | 1 | 0 | 3 | 4 | 13 | 9 | 47 | 19 | 54 | 19 | 54 |
| | 3 | 0 | 0 | 1 | 9 | 5 | 24 | 10 | 77 | 31 | 95 | 30 | 93 |
| | 4 | 0 | 2 | 2 | 6 | 5 | 29 | 12 | 110 | 32 | 130 | 32 | 127 |
| | 5 | 0 | 1 | 1 | 5 | 6 | 9 | 12 | 39 | 18 | 58 | 18 | 57 |
| | 6 | 0 | 0 | 0 | 3 | 3 | 12 | 8 | 44 | 16 | 55 | 16 | 58 |
| | 7 | 0 | 0 | 0 | 4 | 3 | 16 | 9 | 52 | 20 | 61 | 20 | 62 |
| | 8 | 0 | 0 | 2 | 3 | 8 | 20 | 13 | 67 | 27 | 79 | 27 | 78 |
| | 9 | 0 | 0 | 0 | 1 | 2 | 7 | 6 | 25 | 12 | 40 | 12 | 42 |
| | 10 | 0 | 1 | 0 | 2 | 2 | 7 | 7 | 23 | 15 | 29 | 15 | 30 |
| | 11 | 0 | 1 | 3 | 5 | 12 | 21 | 33 | 54 | 43 | 79 | 41 | 79 |
| | 12 | 0 | 0 | 2 | 10 | 9 | 31 | 24 | 114 | 50 | 144 | 50 | 140 |
| | Mean | 0 | 0.5 | 1.0 | 4.4 | 5.5 | 17.0 | 13.0 | 58.5 | 25.7 | 74.2 | 25.4 | 74 |
| | Standard error | 0 | 0.2 | 0.3 | 0.8 | 0.9 | 2.3 | 2.3 | 8.4 | 3.4 | 9.9 | 3.3 | 9 |

[a] The second virus was applied 10 hr after.

TABLE 21. **Lesion counts: The dilution curves of the infectivity of ribonucleic acids respectively isolated from potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean (RKB) and *Chenopodium quinoa*.**

| Local lesion host | Half-leaf no. | Dilution [a] | | | | |
|---|---|---|---|---|---|---|
| | | 0[b] | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ |
| RKB | 1 | 13 | 0 | 0 | 0 | 0 |
| | 2 | 4 | 4 | 1 | 0 | 0 |
| | 3 | 9 | 1 | 0 | 0 | 0 |
| | 4 | 11 | 7 | 1 | 0 | 0 |
| | 5 | 12 | 4 | 2 | 0 | 0 |
| | 6 | 6 | 4 | 0 | 0 | 0 |
| | 7 | 3 | 1 | 0 | 0 | 0 |
| | 8 | 8 | 5 | 1 | 0 | 0 |
| | 9 | 7 | 3 | 1 | 0 | 0 |
| | 10 | 16 | 2 | 2 | 0 | 0 |
| | 11 | 12 | 6 | 0 | 0 | 0 |
| | 12 | 10 | 3 | 2 | 0 | 0 |
| | Mean | 9.3 | 3.3 | 0.8 | 0 | 0 |
| | Standard error | 1.1 | 0.6 | 0.2 | 0 | 0 |
| *C. quinoa* | 1 | 98 | 66 | 30 | 5 | 0 |
| | 2 | 132 | 50 | 17 | 1 | 0 |
| | 3 | 177 | 70 | 22 | 3 | 0 |
| | 4 | 142 | 41 | 16 | 6 | 0 |
| | 5 | 180 | 118 | 13 | 2 | 0 |
| | 6 | 216 | 96 | 26 | 5 | 0 |
| | 7 | 304 | 44 | 14 | 1 | 0 |
| | 8 | 264 | 68 | 9 | 4 | 0 |
| | 9 | 152 | 62 | 21 | 0 | 0 |
| | 10 | 134 | 80 | 13 | 3 | 0 |
| | 11 | 124 | 53 | 15 | 4 | 0 |
| | 12 | 165 | 38 | 11 | 2 | 0 |
| | Mean | 174.0 | 65.5 | 17.3 | 3.0 | 0 |
| | Standard error | 17.4 | 6.8 | 1.8 | 0.5 | 0 |

[a] By using 0.057 M phosphate buffer, pH 8.0.

[b] Concentration: 50 *A* units at *258 nm*.

TABLE 22. Lesion counts: Effect of endogenous ribonuclease associated with intact potato virus S (PVS) on the infectivity of potato virus M (PVM) - ribonucleic acid (RNA) in 'Red Kidney' bean.

| Expt. | Half-leaf no. | Control[a] | Test *(PVM-RNA + PVS)* | | | | |
|---|---|---|---|---|---|---|---|
| | | 45 | 45[b]<br>2.4[c] | 45<br>0.24 | 45<br>0.024 | 45<br>0.0024 | 45<br>0.00024 |
| *I* | 1 | 11 | 3 | 9 | 6 | 11 | 18 |
| | 2 | 5 | 0 | 4 | 4 | 9 | 10 |
| | 3 | 13 | 2 | 3 | 11 | 8 | 8 |
| | 4 | 7 | 2 | 5 | 8 | 6 | 9 |
| | 5 | 9 | 4 | 3 | 5 | 7 | 14 |
| | 6 | 10 | 3 | 6 | 12 | 10 | 6 |
| | 7 | 18 | 4 | 2 | 7 | 17 | 15 |
| | 8 | 8 | 5 | 7 | 6 | 5 | 12 |
| | 9 | 6 | 3 | 4 | 5 | 9 | 7 |
| | 10 | 15 | 1 | 5 | 9 | 14 | 10 |
| | 11 | 7 | 2 | 5 | 4 | 8 | 8 |
| | 12 | 13 | 2 | 4 | 7 | 10 | 9 |
| | Mean | 10.2 | 2.6 | 4.8 | 7.0 | 9.5 | 10.5 |
| | Standard error | 1.1 | 0.4 | 0.6 | 0.8 | 1.0 | 1.0 |
| | | 35 | 35[b]<br>1.6[c] | 35<br>0.16 | 35<br>0.016 | 35<br>0.0016 | 35<br>0.00016 |
| *II* | 1 | 5 | 2 | 2 | 6 | 5 | 6 |
| | 2 | 8 | 1 | 5 | 10 | 8 | 10 |
| | 3 | 9 | 0 | 3 | 3 | 11 | 13 |
| | 4 | 7 | 1 | 6 | 9 | 9 | 5 |
| | 5 | 7 | 1 | 3 | 3 | 7 | 4 |
| | 6 | 6 | 2 | 5 | 4 | 7 | 7 |
| | 7 | 12 | 4 | 4 | 5 | 9 | 6 |
| | 8 | 5 | 3 | 2 | 5 | 5 | 7 |
| | 9 | 3 | 1 | 1 | 8 | 6 | 6 |
| | 10 | 10 | 5 | 4 | 6 | 4 | 10 |
| | 11 | 9 | 3 | 3 | 5 | 8 | 5 |
| | 12 | 8 | 4 | 4 | 7 | 4 | 8 |
| | Mean | 7.4 | 2.3 | 3.5 | 5.9 | 6.9 | 7.3 |
| | Standard error | 0.7 | 0.5 | 0.4 | 0.7 | 0.6 | 0.8 |

[a] PVM-RNA only ( *A* units at *258 nm* ).

[b] *A* units of PVM-RNA at *258 nm*.

[c] PVS (*mg/ml*).

TABLE 23. Lesion counts: Effect of endogenous ribonuclease associated with intact potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in *Chenopodium quinoa*.

| Expt. | Half-leaf no. | Control[a] | Test *(PVS-RNA + PVM)* | | | | |
|---|---|---|---|---|---|---|---|
| | | 10 | 10[b]<br>1.1[c] | 10<br>0.11 | 10<br>0.011 | 10<br>0.0011 | 10<br>0.00011 |
| *I* | 1 | 129 | 58 | 102 | 116 | 142 | 137 |
| | 2 | 54 | 26 | 87 | 90 | 66 | 61 |
| | 3 | 94 | 20 | 44 | 42 | 62 | 75 |
| | 4 | 67 | 27 | 65 | 74 | 72 | 57 |
| | 5 | 104 | 60 | 95 | 95 | 122 | 124 |
| | 6 | 118 | 27 | 74 | 52 | 132 | 104 |
| | 7 | 62 | 26 | 45 | 64 | 59 | 53 |
| | 8 | 97 | 35 | 34 | 65 | 69 | 75 |
| | 9 | 78 | 41 | 68 | 79 | 96 | 128 |
| | 10 | 78 | 45 | 50 | 81 | 50 | 78 |
| | 11 | 97 | 53 | 63 | 68 | 91 | 99 |
| | 12 | 90 | 39 | 38 | 61 | 64 | 94 |
| | Mean | 89.0 | 38.1 | 63.8 | 73.9 | 85.4 | 90.4 |
| | Standard error | 6.5 | 3.9 | 6.5 | 5.8 | 9.0 | 8.3 |
| | | 6 | 6[b]<br>0.7[c] | 6<br>0.07 | 6<br>0.007 | 6<br>0.0007 | 6<br>0.00007 |
| *II* | 1 | 48 | 17 | 50 | 34 | 55 | 62 |
| | 2 | 95 | 36 | 66 | 66 | 99 | 108 |
| | 3 | 118 | 14 | 31 | 110 | 131 | 77 |
| | 4 | 130 | 19 | 28 | 88 | 69 | 129 |
| | 5 | 42 | 13 | 46 | 58 | 48 | 53 |
| | 6 | 43 | 45 | 36 | 44 | 83 | 50 |
| | 7 | 68 | 30 | 80 | 47 | 38 | 70 |
| | 8 | 77 | 56 | 77 | 58 | 45 | 40 |
| | 9 | 50 | 23 | 94 | 90 | 40 | 47 |
| | 10 | 81 | 50 | 41 | 71 | 70 | 87 |
| | 11 | 44 | 40 | 33 | 69 | 108 | 65 |
| | 12 | 58 | 32 | 59 | 98 | 90 | 84 |
| | Mean | 71.2 | 31.3 | 53.4 | 69.4 | 73.0 | 72.7 |
| | Standard error | 8.7 | 4.2 | 6.3 | 6.7 | 8.6 | 7.6 |

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[a] PVS RNA only *( A* units at *258 nm ).*

[b] *A* units of PVS RNA at *258 nm.*

[c] PVM *( mg/ml ).*